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Agricultural Research Institute

PUSA

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VOLUME XVII

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CROPS, PLANT GROWTH, PLANT PRODUCTS, ACTION OF MANURES

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CROPS	
1. " Die Wirkung von Düngemitteln auf den Ernteertrag. Ergebnisseder von Lawes und Gilbert begonnenen Feldversuche an der landwirtschaftlichen Versuchsstation Rothamsted, 1843-1930." E. J. RUSSELL. Archiv Pflanzenbau, Bd. 8, pp. 1-69	1931
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E. John Russell:

Die Wirkung von Düngemitteln auf den Ernteertrag

Ergebnisse der von Lawes und Gilbert begonnenen Feldversuche an der landwirtschaftlichen Versuchsstation Rothamsted 1843 – 1930

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Die Wirkung von Düngemitteln auf den Ernteertrag.

Ergebnisse der von Lawes und Gilbert begonnenen Feldversuche an der landwirtschaftlichen Versuchsstation Rothamsted, 1843—1930.

Von
Sir E. John Russell.

Mit 10 Textabbildungen.

(*Eingegangen am 18. Juli 1931.*)

Die Rothamsteder Versuche wurden begründet von *John Bennet Lawes*, einem englischen Landedelmann, der auf dem schönen alten Herrensitz Rothamsted bei Harpenden lebte. Er hatte das Rittergut 1834 als Sechszehnjähriger geerbt und hatte eine Ader dafür, landwirtschaftliche Versuche anzustellen. Meist war ihm das Glück auch hold, er hatte Erfolg damit. Seine Kenntnisse in Chemie und Pflanzenphysiologie versuchte er zur Verbesserung der Ernteerträge von Feldfrüchten nutzbar zu machen. Zuerst experimentierte er mit Knochendüngung und erzielte wunderbare Erfolge auf englischen Weiden. In Rothamsted selbst freilich versagte dieses Düngemittel. Bald fand er jedoch heraus, daß nach einer Behandlung der Knochen mit Schwefelsäure der gewünschte Erfolg auch hier eintrat, ein Verfahren, durch das eine Umwandlung in sog. „Kalksuperphosphat“ bewirkt wurde. Knochen waren aber teuer, wogegen mineralische Rohphosphate, die damals gerade entdeckt worden waren, niedrig im Preise standen; und so versuchte *Lawes* das gleiche Aufschließverfahren an diesen und zeigte, daß sich tatsächlich aus diesem Ausgangsmaterial eben dasselbe „Kalksuperphosphat“ herstellen läßt wie aus den teuren Knochen. Er erwarb ein Patentrecht darauf und gründete eine Fabrik in der Nähe von London, in der er zum ersten Male ein künstliches Handelsdüngemittel herstellte. Viele Jahre lang lag die gesamte Superphosphaterzeugung in seiner Hand und brachte ihm viel Geld ein.

Es dauerte jedoch nicht lange, da geriet er in Meinungsverschiedenheiten mit einem Manne der Wissenschaft, nämlich mit *Liebig*. *Liebig* hatte 1840 seine epochemachende Schrift: „Die Chemie in ihrer Anwendung auf Landwirtschaft und Physiologie“ veröffentlicht, in der er nachdrücklich betonte, die Pflanze brauche Aschenbestandteile oder „Mineralstoffe“, besonders die Alkalisalze: Kali, Magnesia und Kalk, und behauptete, die Zusammensetzung der Pflanzenaschen gäbe die sichersten Anhaltspunkte für die Düngung von Feldfrüchten ab. Weiterhin erklärte *Liebig*, daß Stickstoffdüngung unnötig wäre, wenn nur die Aschenbestandteile gegeben würden. „Ihren Bedarf an Ammoniak", so sagte er, „werden die Pflanzen ebenso wie den an Kohlensäure aus der Luft decken". — *Lawes* widersprach dieser Auffassung: die Zusammensetzung der Asche gäbe wertvolle Fingerzeige für die Düngung ab — und wandte sich besonders gegen *Liebigs* Ansicht von der Versorgung der Pflanze mit Stickstoff. Er meinte Ernten brauchten Stickstoffdüngung zum mindesten in dem gleichen Maße wie Zufuhr der übrigen Elemente, ja eine N-Gabe steigere den Ertrag sogar mehr als irgendeins der anderen Düngemittel. *Liebig* blieb bei seiner Ansicht und griff *Lawes* Versuche aufs heftigste an. So ging der Meinungsstreit um die Bedeutung der „Mineralstoff"-Theorie bzw. das für und wider eine Stickstoffdüngung eine Reihe von Jahren hin und her.

Lawes fühlte sich der Leitung seiner Superphosphatfabrik und der Durchführung seiner wissenschaftlichen Polemik bald nicht mehr gewachsen. Er nahm daher 1843 den jungen Chemiker *Joseph Henry Gilbert* in seine Dienste und betraute ihn mit der Feldversuchs- und Laboratoriumsarbeit. Es war freilich kaum Laboratorium zu nennen, was er sich da aus einer Scheune hergerichtet und notdürftig ausgestattet hatte, aber es erfüllte seinen Zweck: Viele der grundlegenden Versuche der Wissenschaft vom Landbau sind hier durchgeführt worden. Und es zeigt sich an diesem Beispiel wieder einmal, wie so oft im Werdegang einer Wissenschaft, daß die Anfänge von Männern gelegt worden sind, die unter den schwierigsten Verhältnissen, an notdürftig ausgestatteten Arbeitsstätten und mit primitiven Hilfsmitteln haben arbeiten müssen. Für den sich anschließenden systematischen Ausbau einer Wissenschaft werden derartige Bedingungen freilich unzulänglich, wenn sie auch für die Pionierarbeit auf dem betreffenden Gebiet genügt haben mögen. Im Ausbau der bedeutungsvollen wissenschaftlichen Forschungszweige gibt es ja immer 2 Stufen: Die eine ist das Aufmerksamwerden auf das Sondergebiet, d. h., es werden die ersten grundlegenden Beobachtungen gemacht, was oft unter Zuhilfenahme der einfachsten Mittel geschieht. Die nächste bringt dann die gründlichere Durchforschung des Gebietes, eine Prüfung auf Hieb und Stich; sie ist nur unter Aufwendung peinlichster Genauigkeit zu erreichen. Dieser weitere Ausbau erfordert vorzüglichste Laboratoriumsausstattung, und daraus erklärt es sich, warum es so kostspielig ist, wissenschaftliche Forschungsinstitute auszustatten und zu erhalten.

Lawes' Feldversuche gründeten sich auf seinen Meinungsstreit mit *Liebig*. Sie wurden durchgeführt mit Weizen, Gerste, Kohlrüben (später auf demselben Feld Futterrüben) und einer natürlichen Grasfläche. Sie waren zu dem Zwecke unternommen worden, die Beeinflussung des Pflanzenwachstums durch Stickstoff, Mineralstoffe und Stallmist zu klären. Von Anfang an wurden jedesmal dieselben Früchte Jahr für Jahr auf derselben Versuchsfläche angebaut, ein Verfahren, das ununterbrochen bis zur Gegenwart auf den alten, „klassischen" Feldern Rothamsteds beibehalten worden ist. Nur in einem Falle handelt es sich bei diesen Versuchen um eine Fruchtfolge.

Die Bezeichnungen d. Felder sind:	Ihre Ernten	Dauer der Versuche
Broadbalk-Field	Weizen	1843 bis zur Gegenwart
Barn-Field	Rüben	1843 „ „ „
Hoos-Field	Gerste	1852 „ „ „
	Klee	1849—1853
Agdell-Field	Fruchtfolge	1848 bis zur Gegenwart
Park-Grass	Wiesenheu	1856 „ „ „

Auf den gedüngten Teilstücken der genannten Versuchsfelder (mit Ausnahme von Agdellfield) kommen an *Düngemitteln* zur Anwendung:

1. Stallmist,

2. *Liebigs* „Aschenbestandteile" oder „Mineralstoffe": K, Na, Mg als Sulfate sowie Superphosphat,

3. Nur Stickstoff (als schwefelsaures Ammoniak oder Natronsalpeter),

4. „Mineralstoffe" + Stickstoffdüngemittel,

5. wie 4, doch ohne Kali,

6. wie 4, doch ohne Phosphorsäure (was jedoch nur für den Gerstenversuch auf Hoosfield zutrifft).

Als Vergleich dazu dienen

7. ungedüngte Flächen.

Daneben gibt es in jedem Versuche noch eine Reihe von Teilstücken, auf denen andere Düngemittelkombinationen zur Anwendung gelangen. Die Anzahl der Teilstücke schwankt in den einzelnen Versuchen zwischen 17 und 40.

Der Rothamsteder *Boden* ist ein schwerer Lehm, der reich an Feuersteinen ist.

1876 wurde eine ähnliche Versuchsserie mit Weizen und Gerste 35 km entfernt auf Sandboden in Woburn angelegt. Auch diese Versuche wurden ununterbrochen bis in die heutige Zeit durchgeführt. Einen Überblick über die näheren Einzelheiten dieser beiden Böden und ihre Eigenschaften sowie die klimatischen Verhältnisse, unter denen die Versuche laufen, verschafft Übersicht 1.

Die geringste *Niederschlagsmenge* fällt in Rothamsted im Frühjahr, die größte im Herbst. Ihren Gipfel erreicht die Niederschlagskurve im November. Dies ist jedoch nicht zu allen Zeiten so gewesen. Vielmehr lag er 40 Jahre zurück gegen Ende September und 70 Jahre zurück zu Beginn des September. Es ist wohl möglich, daß gegenwärtig der Höhepunkt wieder rückwärts wandert, und wir wieder in eine Periode mit

Übersicht 1. *Bodenverhältnisse und klimatische Bedingungen der Versuche von Rothamsted und Woburn.*

Teilchengröße in mm	Rothamsted						Woburn		
	Mechanische Bodenanalyse								
	Boden der Harpendener Flur	Barnfield				Broadbalk [Parz. 14]			
	0—10 cm	0—19 cm	19—47 cm	47—97 cm	97—127 cm	8 cm	10—19 cm	19—40 cm	40—63 cm
Grober Sand 2,0 —0,2	9,6	6,7	1,9	2,2	6,4	9,2	39,4	41,2	32,2
Feinsand 0,2 —0,02	39,6	33,0	19,1	13,1	25,0	36,0	29,8	31,9	37,3
Abschlämmbare Teilch.. 0,02—0,002	22,5	18,5	14,3	12,3	15,7	24,0	11,5	12,3	16,5
Ton < 0,002	23,3	31,7	59,3	65,3	49,3	27,0	10,5	10,0	11,7
Feuchtigkeit im lufttrock. Boden	2,0	4,1	6,9	8,4	6,1	2,1	2,9	1,8	1,7
Lösungsverlust	0,8	1,0	0,3	0,2	0,1	0,6	1,0	0,7	0,3
Differenz	+1,3	+5,0	—1,8	—1,5	—2,6	1,1	+4,9	+2,1	+0,3
	100,0	100,0	100,0	100,0	100,0	100,0	100,0	100,0	100,0

	Witterungsverhältnisse und Dränagemenge				
	[Mittel aus 60 Jahren: 1870—1930]				[Mittel aus 48 Jahren: 1882—1930]
	Regenmenge mm	Dranagemenge mm	Dranagemenge in % der Regenmenge	Sonnenscheinstunden (1892—1930)[1]	Regenmenge mm
September	60,0	20,4	34,0	153,7	48,6
Oktober	80,5	46,2	57,4	107,6	65,8
November	72,2	55,1	76,3	65,0	55,3
Dezember	72,9	62,2	85,3	43,9	57,7
Januar	61,5	50,5	82,1	51,8	46,0
Februar	51,6	38,5	74,6	69,3	37,4
März	50,7	27,0	53,3	114,9	42,1
April	51,5	16,7	32,4	157,9	41,9
Mai	52,3	12,1	23,1	204,9	48,6
Juni	56,5	13,7	24,2	204,5	47,5
Juli	69,1	18,2	26,3	202,1	60,4
August	67,3	17,8	26,4	188,8	58,2
Gesamt	746,1	378,4	50,7	1564,4	609,5

niederschlagreicheren Herbstmonaten und trockneren Winter eintreten. Eine ähnliche Verschiebung hat offenbar schon früher einmal stattgefunden, und zwar war dies, den — wenn auch etwas lückenhaften — Aufzeichnungen nach zu urteilen, im 18. Jahrhundert und später in der Mitte des 19. Jahrhunderts der Fall.

Was die Aufrechterhaltung des *Ertrages* anbetrifft, so stellte es sich heraus, daß 1. Weizen, Gerste und Rüben bei Anbau auf ein und demselben Lande befriedigende Erträge liefern, vorausgesetzt, daß regelmäßig Volldüngung verabreicht wird.

2. Die Ertragsleistung geht freilich zurück, sofern nicht eine beträchtliche Überschußdüngung, und zwar in Sonderheit Überschußdüngung an Stickstoff, gegeben wird.

3. Bei Anwendung von künstlichen Düngemitteln zeigen die Erträge größere jährliche Schwankungen als bei Stallmistdüngung.

4. Die gleiche Menge Pflanzennährstoffe im Stallmist wirkt dagegen weniger ertragssteigernd als die entsprechende Menge Nährstoffelement im Handelsdünger.

Die Vergleichswerte für die Dungerleistung sind, je nachdem zu welcher Frucht sie verabreicht worden sind, verschieden. Wird die Leistung von schwefelsaurem Ammoniak = 100 gesetzt, so ergibt sich für

Stallmist bei jährlicher Anwendung

auf dem schweren Rothamsteder Boden: $\begin{cases}\text{zu Weizen} & 40\\ \text{zu Futterrüben} & 62.\end{cases}$

Die *in der Ernte wiedergewonnene N-Menge* beträgt im Durchschnitt für schwefelsaures Ammoniak etwa 50% der angewandten Menge bei Anbau von Getreide und 60% bei Anbau von Futterrüben. Die aus der Stallmistdüngung wiedergewonnene N-Menge beläuft sich dagegen nur auf 20% bei Getreide und rund 35% bei Rüben.

Untersuchungen im Laboratorium haben ergeben, daß etwa 25% des Stallmiststickstoffs innerhalb kurzer Zeit in Nitrate umgewandelt werden. Ein weiterer Bruchteil wird von Mikroorganismen assimiliert und in organischer Form festgelegt, um dann später höchstwahrscheinlich den Pflanzen wieder zugänglich gemacht zu werden.

Weizen, Gerste und Rüben werden mit größerer Rentabilität in Fruchtfolge angebaut als in Einfelderbau. Auch werden dann die Erträge besser auf derselben Höhe erhalten. Die Ursache dafür ist in erster Linie in der bequemeren und gründlicheren Unkrautbekämpfung zu suchen.

Die Ernten.

Allgemeine Ergebnisse.

Weizen: Zu Beginn der Versuche war Weizen in England eine der wichtigsten Feldfrüchte. Daher zog der Versuch mit Weizen auf Broadbalkfield von vornherein die Aufmerksamkeit aller auf sich. Die Aussaat findet in England im Oktober, die Ernte im Juli statt.

Eine Parzelle blieb *ungedüngt* während der ganzen Versuchszeit. Seit 1839 bis zur Gegenwart sind dies 92 Jahre. Im Durchschnitt der ersten 3 Jahre (1843—1846) wurden 17 hl vom Hektar geerntet. Danach fiel der Ertrag allmählich ab. Nach 50 Jahren betrug er noch nahezu 12 hl. Von da ab sank er rascher auf etwa 8. Dabei ist das Aussehen der Pflanzen durchaus normal. Es besteht keinerlei Verstärkung in der Anfälligkeit gegen tierische oder pflanzliche Schädiger. Auch die Kornausbildung ist völlig normal. Trotz 90jähriger, systematischer Aushungerung des Bodens ist es nicht gelungen, der Pflanze das Fortkommen unmöglich zu machen. Freilich läßt das überhandnehmende Unkraut es ihr oft recht schwer werden. Ein Stückchen des Feldes war 1882 vor der Ernte eingezäunt worden. Der Weizen fiel aus und begann unter dem Unkraut zu keimen. 4 Jahre wurde die Fläche sich selbst überlassen. Nach Verlauf dieser Zeit war kein Weizen mehr zu sehen. Seitdem ist er dort nicht wieder hochgekommen. 4 Jahre Kampf mit dem Unkraut unterdrückte ihn also vollständiger als 90 Jahre Raubbau. Und das zeigen alle unsere Versuche: Will ein Landwirt niedrige Erträge haben, so braucht er nur das Unkraut ungestört wachsen zu lassen!

Einige der gedüngten Teilstücke haben bedeutend höhere Erträge gebracht. Die mit den *Aschenbestandteilen* (gemäß der Liebigschen Theorie) versehenen freilich nicht. Sie brachten kaum 1 hl/ha mehr als die ungedüngten.

Wird dagegen obendrein ein Stickstoffdüngemittel verabfolgt, dann steigt der Ertrag mit einem Schlage ganz erheblich, und zwar um 7,4 hl für die erste Gabe von 50 kg/ha N (=2,5 dz. schwefelsaurem Ammoniak), 15,2 hl für die 2., dagegen nur weitere 3,6 hl für die 3. Daraus geht hervor, *daß* eine *höhere Stickstoffdüngung unter Umständen rentabler sein kann als eine niedrigere.* Ein Landwirt kann also ebensogut Geld verlieren, wenn er nicht genügend düngt, wie wenn er zuviel gibt.

Der Weizen scheint demnach in erster Linie einen hohen Bedarf an Stickstoff zu haben. Andere Versuche haben nun gezeigt, daß Natronsalpeter besser ist als schwefelsaures Ammoniak. Eine zu hohe Stickstoffgabe ist nicht nur unrentabel, sie verändert auch den Habitus der Pflanze ganz erheblich. Die Blätter werden breiter und länger, viel dunkler in der Farbe und anfälliger für Rost. Blätter und Ähre werden für den Halm zu schwer, so daß der Weizen lagert. Der unterste Halmknoten ist die empfindlichste Stelle. Die Halmfestigkeit setzt also der Anwendung von Stickstoffdüngung bezüglich Ertragssteigerung eine Grenze, wogegen vorderhand kein sicheres Gegenmittel bekannt ist. Kalidüngung übt allerdings bis zu einem gewissen Grade eine Gegenwirkung aus, doch darauf werden wir später zu sprechen kommen.

Eins der bemerkenswertesten Ergebnisse des Versuches ist die *Wirkung von Brache.* 1926, also nach der 74. Ernte, wurde ein Teil des Feldes 2 Jahre gebracht, wobei allerdings eine energische Unkrautbekämpfung mit der Egge vorgenommen wurde. Im Oktober 1927 wurde wieder wie gewöhnlich Weizen ausgesät. Der Weizen brachte im folgenden Jahre nicht nur hohe Erträge, sondern auch das Hektolitergewicht war ungewöhnlich hoch und der Kornanteil an der Gesamternte war viel höher als sonst. So brachte die seit 1839 ungedüngte Fläche nicht weniger als 25 hl/ha Korn, wogegen die ungebrachte Hälfte nur 6,3 hl/ha abwarf. Auf den Volldüngungsparzellen wurden über 50 hl, d. h. mehr als das Doppelte des Ertrages der nicht gebrachten Hälfte geerntet. Es ist noch nicht ganz sicher, wie diese gewaltige Erntesteigerung zu erklären ist. Jedenfalls scheint sie nicht einfach die Folge von Stickstoffanhäufung im Boden während des Brachejahres zu sein. Dieser günstige Einfluß ist aber nur vorübergehend, er hält nicht länger als 1 Jahr vor. Wir sind im Augenblick gerade damit beschäftigt, diesen Fragen etwas mehr nachzugehen.

Übersicht 2. *Broadbalkfield, Rothamsted. Wirkung von 2 Jahre Brache auf den Weizenertrag (1926—1927 Brache).*

Parz.-Nr.		Korn hl/ha			Stroh dz/ha		
		1928	1929	77 jähr. Durchschnitt	1928	1929	77 jähr. Durchschnitt
3	Ungedüngt seit 1839 . . .	25,1	8,2	10,6	34,9	13,9	12,4
13	Mineralische Volldüngung .	49,7	23,0	26,3	70,8	44,8	38,7
11	Ohne Kali.	51,2	17,1	19,3	72,6	36,6	27,4
10	Ohne Kali u. Phosphorsaure	42,3	22,2	16,9	54,0	42,2	22,7
5	Ohne Stickstoff	31,7	8,2	12,2	43,7	11,8	14,6
2 B	Stallmist	43.6	27.0	29.9	77.1	50.4	43.3

In England wünschen die Mühlen einen Weizen mit hohem Stickstoffgehalt. Durch stärkere Stickstoffgaben wird der prozentuale Stickstoffgehalt des Kornes nun zwar erhöht, jedoch nicht in genügend großem Ausmaß, um den Marktwert der Ware zu erhöhen. Wir haben daher auch Versuche mit anderen Düngungen gemacht, um den Stickstoffgehalt des Weizens und somit seinen Marktwert zu erhöhen, der gewünschte Erfolg ist aber vorläufig noch ausgeblieben.

Gerste: Die Versuche mit Sommergerste laufen auf *Hoosfield* seit 1852. Sie wurden 10 Jahre später als die Weizenversuche auf Broadbalk angelegt. Die Aussaat von Sommergerste erfolgt in England im Februar und März, die Ernte fällt nach der Weizenernte.

Die Ergebnisse des Versuches ähneln denen des Weizenversuches: Sie zeigen, daß Stickstoff der wesentlichste Faktor ist. Eine Gabe von

50 kg/ha N hat bei Gerste etwa die gleiche ertragssteigernde Wirkung wie bei Weizen. Ammoniumchlorid ist bei Gerste dem schwefelsauren Ammoniak vorzuziehen.

Ein Unterschied besteht zwischen den beiden Getreidearten hinsichtlich ihres Bedarfs an Phosphorsäure. Dies macht sich ganz besonders in Rothamsted da bemerkbar, wo der Boden sehr schwer ist. Während der Jugendentwicklung beschleunigt Phosphorsäure das Wurzelwachstum und fördert die Bestockung. In späteren Entwicklungsstadien der Pflanze beschleunigt sie den Reifeprozeß.

Kalidüngung hat für die Entwicklung der Gerste keine große Bedeutung, ja unter Umständen drückt sie den Ertrag sogar etwas. Auf die Kornausbildung hat sie dagegen einen sehr günstigen Einfluß, indem sie die Qualität verbessert. Etwa 65 % der englischen Gerste geht in die Mälzereien. Diese verlangen einen niedrigen Stickstoffgehalt der Gerstenkorns. Der N-Gehalt soll 1,3—1,4 % nicht überschreiten, denn bei höherem Gehalt leidet die Qualität des extrahierbaren Malzes, und es entstehen Schwierigkeiten beim Brauen. N-Düngemittel erhöhen nun zwar den Ertrag an Korn, haben aber auch nur zu leicht erhöhten N-Gehalt desselben und damit geringere Brauqualität zur Folge. Kalidüngesalze dagegen drücken den N-Gehalt des Kornes und verbessern somit dessen Eigenschaften zu Brauzwecken. Freilich reicht diese qualitätsverbessernde Wirkung nicht immer aus, um auch den Preis zu beeinflussen. Immerhin kann es für den Käufer bei großem Angebot ein Anreiz für seine Auswahl der am Markt befindlichen Ware bedeuten. Doch haben Kalidüngemittel noch eine andere Bedeutung: In England wird gewöhnlich Klee in die Gerste eingesät, und Klee lohnt eine Kalidüngung sehr. Seine Winterfestigkeit wird durch Kali gesteigert. Diese beiden Gründe sprechen also für eine Kalidüngung zu Gerste, wenn eine N-Gabe verabfolgt wird.

Futterrüben: Die Versuche wurden auf *Barnfield* zunächst mit *Kohl-* und *Wasserrüben* durchgeführt, weil diese in der Zeit, als die Versuche angelegt wurden, eine große Bedeutung für England hatten. Auch heute sind sie noch wichtig für Nordengland, wenn sie auch für den Süden immer mehr an Bedeutung verlieren. Immerhin werden auch heute noch große Flächen damit bestellt und gleich auf dem Felde an Schafe verfüttert.

Hauptbedarf haben diese Pflanzen nicht an Stickstoff sondern an Phosphorsäure, für die sie wahrscheinlich ein schlechtes Aufnahmevermögen besitzen. Der Bedarf an diesem Nährstoff ist an und für sich nicht so stark, wenn man bedenkt, wie gering ihr Gehalt an Phosphorsäure ist. Aber seine Aufnahme ist erleichtert, wenn er sich in löslicher Form im Boden befindet. Die wichtigste Wirkung einer Superphosphatgabe besteht in der Beschleunigung des Wachstums während der Jugendentwicklung der Rüben, die zur Folge hat, daß die Rüben früher gehackt werden können. Auch die Entwicklung der Rübenkörper wird dadurch gefördert. Kohlrüben sind übrigens außerordentlich empfindlich gegenüber Witterungseinflüssen. Wenn die Bodenverhältnisse und die klimatischen Bedingungen Ernten von über 350 dz/ha garantieren, ist Stickstoffdüngung am Platze. Wo mehr als 800 dz/ha geerntet werden können, empfiehlt sich Volldüngung und Stallmist. Unter günstigen Bedingungen sind Ernten von 1250 dz/ha im Norden Englands erzielt worden. In Rothamsted betrug die Höchsternte allerdings nur 750 dz/ha.

Seit 1876 werden Versuche mit *Runkelrüben* auf eben demselben Felde gemacht. Die Runkel ist heutzutage eine ziemlich beliebte Futterpflanze in den Viehwirtschaften Südenglands. Im Norden kommt sie kaum fort, es ist dort zu kalt. Wir säen sie im April aus und ernten sie im Oktober vor dem ersten Frost. Mit dieser Rübenart werden die größten Massenerträge erzielt. 1000 dz/ha ist noch nichts Besonderes, es werden gelegentlich in manchen Gegenden Ernten von 2000 dz/ha gemacht. Natürlich ist für solche gewaltigen Leistungen starke Düngung erforderlich.

N-Düngung veranlaßt die Pflanze zu verstärkter Stickstoffaufnahme. Soweit Untersuchungen darüber vorliegen, ist die Aufnahme etwa proportional der Zufuhr. Erhöhte N-Zufuhr hat zwar eine Vergrößerung der Blattmasse und der assimilierenden Blattoberfläche zur Folge, drückt aber die assimilatorische Nutzleistung herab. Das Ergebnis ist daher ein ständiges Anhäufen von N in der Pflanze, ohne daß entsprechende Mengen Kohlehydrate gebildet würden. Der tatsächliche Ertragszuwachs ist verhältnismäßig klein und die Pflanzen bekommen ein ungesundes Aussehen.

Eine Beigabe von Kalisalzen erhöht die Wirksamkeit des Blattes und den Ertrag, ohne die Blattgröße wesentlich zu beeinflussen. Phosphate haben keine erhebliche Ertragssteigerung auf den Rothamsteder Böden bewirkt, obwohl auf anderen englischen Böden, z. B. auf der Versuchswirtschaft Saxmundham, wesentlich bessere Erfolge damit erzielt worden sind. Auf den dortigen Versuchsfeldern kann man die typischen Erscheinungen von Phosphorsäuremangel ganz herrlich verfolgen.

Kartoffeln: Als 1843 die Rothamsteder Versuche angelegt wurden, spielten Kartoffeln keine große Rolle unter den Feldfrüchten. 1876 wurden die ersten Versuche damit gemacht, die dann 1901 abgebrochen worden sind. 1914 wurde eine neue Versuchsserie ausgelegt, die allmählich erweitert worden ist. Die gegenwärtige Form bekam der Anlageplan im wesentlichen 1926. 2 Jahre später wurden nur noch einige kleine Veränderungen vorgenommen. Der Versuch umfaßt die Prüfung von Stickstoff-, Kali- und Phosphorsäuredüngemitteln. Die beiden ersteren werden in steigenden Gaben verabreicht.

Übersicht 3. *Long Hoosfield 1930. Durchschnittlicher Ertrag an Kartoffeln. dz/ha.*

Düngung	Ohne Superphosphat			Mit Superphosphat		
	ohne N	+ 1,25 dz/ha Schwefelsaures Ammoniak	+ 2,5 dz/ha Schwefelsaures Ammoniak	ohne N	+ 1,25 dz/ha Schwefelsaures Ammoniak	+ 2,5 dz/ha Schwefelsaures Ammoniak
Ohne Kali	190	204	221	198	209	245
+ 0,5 dz/ha K_2O .	192	234	226	208	247	255
+ 1,0 dz/ha K_2O .	202	239	232	222	258	276
Form der Kaligabe	Schwefelsaures Kali	Salzsaures Kali	50% Kali-Düngesalz	Schwefelsaures Kali	Salzsaures Kali	50% Kali-Düngesalz
Ohne Kali	204	204	204	217	217	217
+ 0,5 dz/ha K_2O .	236	203	212	232	236	242
+ 1,0 dz/ha K_2O .	225	227	220	255	262	238

$\sigma = 5{,}4$ dz/ha.

Zuckerrüben: Zu 3 verschiedenen Zeitpunkten sind Versuche mit Zuckerrüben unternommen worden: Das erstemal 1871—1873, dann 1898—1901 und endlich 1927 bis zur Gegenwart. In allen Fällen stammte das Saatgut vom Kontinent. Verglichen mit den Ernten vor 60 Jahren sind die Rüben viel zuckerreicher geworden, im Rüben- sowohl wie im Zuckerertrag auf die Flächeneinheit sind sie dagegen zurückgegangen, mit anderen Worten, die assimilatorische Wirksamkeit des Blattapparates hat sich bedeutend verringert. Offensichtlich besteht die Verbesserung hauptsächlich in Verringerung der Rübenmasse, wodurch die Zuckermenge auf einen kleineren Raum zusammengedrängt worden ist.

	Ertrag in dz/ha		Auf 1 dz Blattschopf entfallen Rüben	Zucker in den Rüben	
	Rüben	Blatt	dz/ha	%	dz/ha
1871—1873	475	128	3,7	11,0	52,2
1898—1901	337	166	2,0	13,7	46,1
1926—1930	226	312	0,7	17,8	40,3
Die entsprechenden Daten für Futterrüben sind:					
1926—1930	565	126	5,0	—	—

Im Durchschnitt wird durch 1 dz/ha schwefelsaures Ammoniak eine Mehrernte von 24 dz/ha Blatt und 12 dz/ha Rüben erzielt, während der Zuckergehalt um 0,05 % gedrückt wird. Offenbar bestehen hier Aussichten auf beträchtliche Verbesserung, sowohl was Sorten betrifft, wie auch was die Kultur der Rüben angeht. Die mangelhafte Ausnutzung von Düngung scheint jedenfalls darauf hinzuweisen, daß in den gegenwärtig gebräuchlichen Sorten eine Art Verarbeitungsstockung auf Grund innerer Ursachen auftritt, die noch beseitigt werden muß.

Stickstoffdüngemittel haben wie gewöhnlich Vermehrung der Blattmasse zur Folge. Über eine gewisse Grenze hinaus vergrößern sie das Rübengewicht dagegen nicht, wohl aber drücken sie den relativen Zuckergehalt ein wenig. Die Wirkung von Kalidüngesalzen tritt nicht so deutlich zutage wie bei Futterrüben. Beidüngung von gewöhnlichem Kochsalz ist vorteilhaft. Weitere Versuche sind im Gange zur Klärung der Frage nach der Wirkung seiner beiden Bestandteile: Natrium und Chlor. Wie es scheint sind beide von Bedeutung.

Gemischte Bestände.

Futtergemenge: Für die Düngeweise von Mischbeständen wie Futtergemenge und Wiesen gelten ganz andere Gesichtspunkte wie für Einzelkulturen. Jedes beliebige Düngemittel, das verabfolgt wird, hat die Aussicht, einen Bestandteil des Mischbestandes mehr zu fördern als die anderen und somit seine Entwicklung und seine Chancen im Wettstreit mit den anderen zu verbessern. Denn die gut genährten Pflanzen überwuchern den Restbestand und verdrängen ihn allmählich.

Dies zeigt sich sehr schön bei den Versuchen mit Futtergemengen. Die englischen Landwirte bauen Gemenge von Getreidearten und Leguminosen zu Futterzwecken an. Diese werden grün geschnitten zur Silagebereitung oder sie werden zu Heu gemacht. Auch läßt man sie reif werden und drischt sie dann aus, um Korn und Stroh getrennt zu verwerten. Das ist in den einzelnen Wirtschaften verschieden.

Häufig verwendet man folgende Mischung:

1 hl/ha Pferdebohnen (Vicia Faba)
2 hl/ha Saatwicken (Vicia sativa) oder Erbsen
2 hl/ha Hafer oder Gerste.

Es hat sich gezeigt, daß Kali- und Phosphorsäuredüngung die Erträge steigert, eine Stickstoffdüngung dagegen, besonders wenn sie in Form von schwefelsaurem Ammoniak verabreicht wird, die Leguminosen im Bestand unterdrückt und somit den Futterwert der — wenn auch vermehrten — Erntemasse keineswegs erhöht.

Wiesenbestände: Die Rothamsteder Versuche mit Grasbeständen werden vorwiegend als Heu geerntet. Versuche mit Abweiden der Grasparzellen sind von Zeit zu Zeit immer einmal wieder gemacht worden, die Technik war jedoch nicht befriedigend.

Das Versuchsland hat seit Jahren unter einer Grasnarbe gelegen, wahrscheinlich sogar seit Jahrhunderten. 1856 wurde es in Streifen aufgeteilt, von denen jeder eine besondere Düngung erhielt. Bei einigen dieser Streifen ist die ursprüngliche Behandlungsweise ununterbrochen beibehalten worden, bei anderen sind Änderungen eingetreten. In allen Fällen wurde die Veränderung in der Zusammensetzung des Bestandes sorgfältig beobachtet.

Übersicht 4. *Heuertrag und seine Zusammensetzung eines Futtergemenges.*

Düngung: N dz/ha	Ungedüngt	0.25	0,50
Geerntete Trockensubstanz dz/ha:			
Hafer-Wicken-Gemenge	27,51	40,32	40,69
Hafer-Erbsen	32,66	39,31	42,83
Gerste-Wicken	34,29	38,56	47,23
Gerste-Erbsen	32,78	41,45	48,86
Mittel	31,78	39,94	44,96
Zusammensetzung der Trockensubst. in Proz.:			
Protein	11,7	9,6	8,6
Lösliche Kohlehydrate	46,2	48,8	49,1
Pflanzenfaser	32,9	32,5	33,4
Fett	2,4	2,6	2,5
Asche	6,8	6,5	6,4
Leguminosenanteil am Ertrag in Prozenten	41,0	27,0	20,0
Leguminosen: Trockensubstanz: dz/ha	12,94	10,93	9,04
Getreidepflanzen: Trockensubstanz: dz/ha	18,97	29,01	35,92
N in der Erntemasse: dz/ha	0.53	0.55	0.55

Zusammensetzung von Wiesenheu (nach T. B Wood).

	Sehr gut	Gut	Schlecht
Protein	16,1	11,3	8,8
Lösliche Kohlehydrate	48,2	47,9	44,6
Pflanzenfaser	23,0	30,7	39,1
Fett	3,6	2,9	1,8
Asche	9.2	7.2	5.8

Die ungedüngten Flächen sind zunehmend magerer geworden infolge des ständigen Aberntens ihres Bestandes. Diese Ausmergelung äußert sich in zweierlei Weise: Der Ertrag ist gefallen, die Anzahl der Arten hat sich dagegen stark erhöht. Auf solchen Mangelparzellen vermögen, wo die übrigen Wachstumsbedingungen erfüllt sind, Pflanzenarten zu überdauern, die sonst unterdrückt werden. 50 verschiedene Arten kommen auf den ungedüngten Flächen vor.

Auf den gedüngten Flächen ist die Anzahl der Arten viel kleiner. Zuerst gingen aus: Das Zittergras (Briza media) und der Hornklee (Lotus corniculatus). Vorkommen dieser Pflanzen kann somit geradezu als Anzeichen für Nährstoffmangel angesehen werden, der Überwucherung durch üppiger wachsende Pflanzen verhindert. Welche Arten sich halten, das hängt von der Versäuerung des Bodens und der Nährstoffzufuhr ab: Ein saurer Boden ist ungeeignet für Leguminosen, sie werden infolgedessen verdrängt. Stickstoffdüngung fördert die Entwicklung von Gräsern und anderen Nichtleguminosen. Kali- und Phosphorsäuregaben bei Fehlen von Stickstoff begünstigen die Leguminosen, ohne andere Pflanzen zu unterdrücken, weil die Leguminosen sie mit Stickstoff versorgen. Volldüngung bei ausreichender Kalkung erhält Leguminosen und Nichtleguminosen in gleicher Weise im Bestand. Alleinige Anwendung von schwefelsaurem Ammoniak schafft eine vorwiegend aus feinen Gräsern zusammengesetzte Narbe, wie man sie in Anlagen findet, was übrigens eine Folge von Versäuerung des Bodens ist. Einseitige Bedüngung mit Natronsalpeter fördert dagegen die Verbreitung von Kräutern, ist also ungeeignet für Zierrasen und auch für landwirtschaftlich genutzte Grünlandflächen.

Die auf den verschieden gedüngten Teilstücken vorherrschenden Pflanzenarten sind: auf den Flächen ohne Stickstoff mit *Kali und Phosphorsäure*, Kalk nach Bedarf: Wiesenblatterbse (Lathyrus pratensis), Rotklee (Trifolium pratense), Schafschwingel (Festuca ovina), Knaulgras (Dactylis glomorata), Straußgras (Agrostis vulgaris), Ruchgras (Authoxanthum odoratum), Honiggras (Holcus lanatus). Ferner Flockenblume (Centaurea nigra) und Schafgarbe (Achillea millefolium).

Fehlen von Kalidüngung verringert den Anteil, den Dactylis am Bestande hat, und bringt dafür die Horste von Holcus zur Entfaltung. Auch der Rotklee wird verdrängt.

Fehlt der Kalk, so wird Alopecurus, der Wiesenfuchsschwanz, von Anthoxanthum verdrängt, der Gräseranteil überhaupt nimmt ab, der Klee geht allmählich ganz aus und krautartige Gewächse breiten sich stärker aus. — Stickstoff im Verein mit Phosphorsäure und Kalidüngemitteln (*Volldüngung*) beeinflußt die Narbe je nach der Form des N-Düngemittels: a) auf den alkalischen Böden der mit Natronsalpeter behandelten Flächen finden sich in größerer Menge: Alopecurus pratensis, Arrhenatherum avenaceum, Dactylis glomorata, Festuca ovina, Avena pubescens, in manchen Jahren auch Bromus mollis, Lathyrus pratensis, Taraxacum vulgare und Anthriscus ein. Im ganzen sind es etwa 16 bis 25 Arten, deren Anteil in den einzelnen Jahren schwankt. Die geringere Zahl gilt für die stärker gedüngten Flächen. b) Mit dem bodenversauernden schwefelsauren Ammoniak fördert man im Bestand (bei Verabfolgung reichlicher Gaben): Holcus lanatus, Arrhenatherum avenaceum, Agrostis vulgaris, Anthoxanthum odoratum, Festuca ovina, Rumex. (Durch schwächere Gaben werden einige andere Arten nicht ganz oder nicht so rasch verdrängt.)

Bei *Fehlen von Kali* breitet sich Festuca, in manchen Jahren auch Anthoxanthum stärker aus, Rumex tritt dagegen zuweilen fast ganz zurück, Holcus und Arrhenatherum sind viel weniger verbreitet.

Bei *einseitiger Stickstoffdüngung* a) mit Natronsalpeter setzt sich der Bestand zu etwa gleichen Teilen aus Gräsern und anderen Nichtleguminosen zusammen. Die etwa 35 Grasarten ändern sich anteilsmäßig wenig, nur Dactylis nimmt zu. Von den krautartigen Gewächsen breiten sich Leontodon hispidus (Löwenzahn) und Plantago lanceolata (Spitzblättriger Wegerich) immer mehr aus. Leguminosen sind selten (Lathyrus, Lotus, Trifolium). b) Einseitig mit schwefelsaurem Ammoniak gedüngte Flächen liefern fast ausschließlich Gräser in der Ernte. Unter den vorkommenden 6 Grasarten sind die wichtigsten: Festuca ovina, Agrostis vulgaris, Anthoxanthum, Holcus, Dactylis. Außerdem bleiben Centaurea nigra, Rumex, Conopodium denudatum zu erwähnen.

Für den Landwirt sind folgende Ergebnisse von praktisch-wichtiger Bedeutung: Mineralische Volldüngung gewährleistet bei in Abständen wiederkehrender Anwendung von Kalk auf die Dauer hohe Heuerträge von befriedigender Güte. Bessere Leistungen werden erzielt, wenn an Stelle der Volldüngung alle 4 Jahre einmal Stallmist gegeben wird. Auslassen von Kali aus der Düngung führt zu Ertragsnachlaß und vermindert den Kleeanteil. Auslassen von Stickstoff (einseitige Mineralstoffdüngung) vermehrt den Leguminosenanteil, mithin den Futterwert, allerdings auf Kosten der Erntemenge. Fehlen von Kalk wirkt sich bei uneingeschränktem Gebrauch von schwefelsaurem Ammoniak sehr ungünstig aus: die Leguminosen verschwinden allmählich ganz, und es kommt zu einer einseitigen Förderung der Gräser. Es kommt zwar zu hohen Massenerträgen, die Qualität des Heues ist aber minderwertig. Abb. 1 gibt noch einmal einen Überblick über die Ergebnisse.

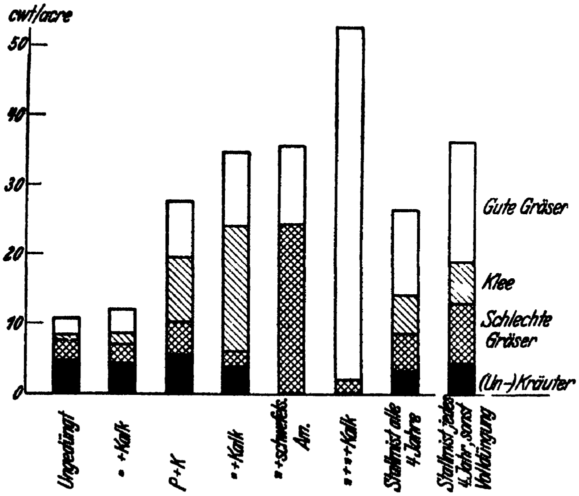

Abb. 1.

In letzter Zeit sind diese Ergebnisse mit viel Erfolg auf Weideland angewandt worden. Bis vor kurzem war Thomasmehl (Basic slag) das übliche Düngemittel für Weiden auf Grund seiner günstigen Beeinflussung der Leguminosen. Viele der Weiden auf den Tonböden Englands sind nur dürftig mit armen Gräsern und kleinen Kleearten bestanden und zeigen viele Kahlstellen. 7—12 dz Thomasmehl auf das Hektar schaffen eine wunderbare Umwandlung: Wilder Weißklee wächst plötzlich hervor und füllt bald die Lücken aus; eine beträchtliche Verbesserung des Weidewertes der Fläche ist die Folge. Wahrscheinlich erklärt sich der Vorgang daraus, daß Phosphate die Entwicklung der Knöllchenbakterien von der unbeweglichen Form in die bewegliche beschleunigen und dadurch die Infektion der Kleewurzeln erleichtern. Außerdem wird durch die Versorgung des Bodens mit Phosphorsäure der Phosphatgehalt der Ernte erhöht, der für die Tierernährung von großer Bedeutung ist. Der Phosphorsäuregehalt der Futterpflanzen ist auf der ganzen Welt meist so niedrig, daß er für die Tiere zur Deckung ihres Bedarfs nicht ausreicht, sondern zu krankhaften Mangelerscheinungen führt (S. 31). Die Wirkung von Phosphorsäuregaben auf den Bestand ist dargestellt in Übersicht 5.

Übersicht 5. *Einfluß von Phosphorsäuredüngung auf den Ertrag und die Zusammensetzung von Wiesenheu.*

	Ertrag	In % der Trockensubstanz		Im Heu enthalten: dz/ha		Durchschnittlicher Anteil der in der Ernte wiedergewonnenen Düngungs-P_2O_5
	dz/ha	N	P_2O_5	Eiweiß	P_2O_5	%
Superphosphat	40,4	1,73	0,61	4,46	0,244	5,8
Thomasmehl: 96,5%[1] .	38,6	1,55	0,53	3,71	0,206	2,7
23,0% .	38,2	1,49	0,49	3,44	0,190	1,5
Rohphosphat	37,7	1,51	0,49	3,36	0,186	1,2
Ungedüngt	36,7	1,48	0,47	3,32	0,171	—

Neuerdings macht man in der Praxis auch Gebrauch von Stickstoffdüngung auf Weideland. Die wuchsfördernde Eigentümlichkeit dieser Düngemittel kommt besonders den Milchwirtschaften sehr zustatten. Allgemein wird frühzeitiges Austreiben der Tiere im Frühjahr sowie Verlängerung der Weidezeit in den Herbst hinein durch sie ermöglicht. Auf diese Weise wird der Landwirt in den Stand versetzt, die billigen N-Düngemittel zur Ernährung seines Viehs zu verwenden, anstatt teure Kraftfuttermittel anschaffen zu müssen. In einer englischen Viehwirtschaft wurden mit einer Düngung von 5 dz/ha schwefelsaurem Ammoniak (in 4 Teilgaben verabfolgt), 5 dz/ha Kainit und 6,3 dz/ha Superphosphat folgende Ergebnisse erzielt[2].

	Ohne Stickstoff	Schwefelsaures Ammoniak
Milchertrag l/ha	4594	8009
Austrieb	Mitte Mai	Erste April-Woche
Weidedauer	150 Tage	185 Tage
Erforderliche Weidefläche je Kuh .	0,34 ha	0,28 ha

---

[1] der Gesamt-P_2O_5 löslich in 2% Citronensäure.

[2] *W. Brunton*, 1929 Farmers Club, S. 1.

Schwefelsaures Ammoniak kommt hauptsächlich zu solchen Zwecken zur Verwendung. Die Gefahr, die Leguminosen bei Gebrauch dieser N-Form zu verdrängen, ist aber sehr groß. Mehr als man im Frühjahr gewinnt, kann im Herbst wieder verlorengehen.

Der Einfluß von Düngemitteln auf das Pflanzenwachstum.

Lange Zeit war das Interesse an den Versuchen ausschließlich auf die Beeinflussung der Ertragsbildung durch Düngung gerichtet. Heutzutage tritt man mit einer erweiterten Fragestellung an diese heran: Wie werden Pflanzenwachstum und Zusammensetzung der Ernte durch die einzelnen Nährstoffelemente beeinflußt, so fragt man, und ist nicht allein an dem Endeffekt, der Ertragsleistung, interessiert. Dazu kommt, daß man den Einfluß von Boden und Witterung losgelöst von den übrigen Fragen zu ermitteln trachtet.

Stickstoff-Düngemittel.

Die älteren Landwirte glaubten, Ammoniak sei die eigentliche Nährstofform für die Pflanzen. 1860 wurde dann zum ersten Male in Frankreich von Chemikern die Ansicht vertreten, die Pflanze nehme den Stickstoff vorwiegend in Form von Nitraten auf. Gegenwärtig kehrt man in der Pflanzenphysiologie wieder zu der ursprünglichen Auffassung zurück, gibt indessen zu, daß Nitrate leichter aufgenommen werden. Nur müssen sie innerhalb der Pflanze erst reduziert werden, ehe sie zur Weiterverarbeitung dienen können.

Die physiologische Wirkung des Stickstoffs besteht in Vergrößerung der Blattfläche. Nicht vergrößert wird dagegen der assimilatorische Effekt je Flächeneinheit, den wir als „Nutzleistung“ des Blattapparates bezeichnen wollen. Infolge der Vergrößerung der Blattfläche kommt es dabei doch zu erhöhtem Endeffekt der assimilatorischen Tätigkeit der Pflanze. Unter Rothamsteder Bedingungen werden folgende Durchschnittsergebnisse erhalten: Auf die Einheit absorbierten Stickstoffs werden an Einheiten Trockensubstanz gebildet von

Weizen . . .	80	Futterrüben .	75	Wiesenheu . .	60
Gerste	80	Kohlrüben . .	40	Rotklee . . .	40
		Kartoffeln . .	70		

Die Pflanze nutzt andererseits von der in der Düngung gegebenen Stickstoffmenge nur etwa 50—70 % aus, der Rest verbleibt entweder im Boden oder geht verloren. Rechnet man die in 1,25 dz/ha schwefelsaurem Ammoniak enthaltene N-Menge als Stickstoffeinheit — eine Gabe wie sie in England üblicherweise verabfolgt wird — so entfällt auf eine Stickstoffeinheit bei den einzelnen Feldfrüchten folgende Steigerung des Ertrages:

	Frischgewicht	Trockengewicht
Kartoffelknollen	100	23
Weizenkorn	15	13
Weizenstroh	25	20
Gerstenkorn	15	13
Gerstenstroh	18	15

Der Ertragszuwachs ändert sich natürlich entsprechend den Witterungsverhältnissen. Die Schwankungen sind jedoch nicht so erheblich. Die 2. Stickstoffeinheit gibt größeren Ertragszuwachs als die 1., bei der 3. und weiteren Gabe nimmt der Zuwachs dagegen gewöhnlich wieder ab. So haben sich z. B. in einem Versuch mit Kartoffeln durch steigende N-Gaben bei reichlicher Zufuhr der übrigen Nährstoffe folgende Werte ergeben:

Übersicht 6. *Erntezuwachs je Stickstoffeinheit (Kartoffeln, dz/ha).*

	− N	+ 1 N	+ 1 $^1/_2$ N	+ 2 N	+ 3 N	+ 4 N
1926[1]	195,7	+ 30,6	.	+ 36,0	.	+ 23,8
1927[1]	163,5	.	.	+ 12,8	.	±
1928[2]	192,7	.	+ 22,7	.	+ 33,9	.
1929[2]	122,8	.	+ 14,7	.	+ 12,4	.
1930[1]	222,0	+ 36,0	.	+ 18,0	.	.

Die Beziehungen zwischen Erntemenge und Düngeraufwendung auf eine Formel zu bringen, ist verschiedentlich versucht worden. *Mitscherlich* war einer der ersten, der quantitative Beziehungen aufzustellen versuchte. Seine Untersuchungen spornten zu zahlreichen gleichartigen Versuchen an. Die erste seiner Formeln war eine einfache logarithmische Gleichung. Sie erwies sich jedoch als zu einfach und wurde daher von ihm selbst, *Bondorff* u. a. modifiziert. In Rothamsted benutzen wir eine mehr allgemeine Formel, die sich auf Fälle mit 2 oder mehr Variablen anwenden läßt. Sie ist hervorgegangen aus der Formel, die *Maskell* für Widerstandsmessungen aufgestellt hat:

$$\frac{1}{y} = F(\mathrm{N}) + F_1(\mathrm{K}) + F_2(\mathrm{P}) + \cdots,$$

wobei y = Ertrag, $F\ (\mathrm{N}) = \frac{a_n}{n + N}$ bedeutet. (Im letzteren Ausdruck ist n = Menge des betreffenden Düngeelementes im Saatgut und Boden, N = Menge im Düngemittel und a_n = eine Konstante.)

Diese Formel gibt befriedigende Ergebnisse zur Errechnung von Versuchsergebnissen, wie nachstehender Vergleich zeigt:

---

[1] Der N-Einheit entsprach eine Menge von 1,25 dz/ha schwefels. Ammoniak.

[2] Der N-Einheit entsprach eine Menge von 1,87 dz/ha schwefels. Ammoniak.

Übersicht 7. *Ertrag von Gerste. Trockensubstanz je Gefäß.*
(Nach *F. G. Gregory.*)

P_2O_5-Gabe je Gefäß mg	N-Gabe je Gefäß (mg)		$\frac{y}{y_1}$	$\frac{1}{y}-\frac{1}{y^1}$
	15	1215		
	y	y_1		
5	3,06	15,00	4,902	0,2602
15	3,36	17,88	5,321	0,2417
45	3,03	29,60	9,769	0,2963
135	3,39	68,10	20,089	0,2784
405	3,60	86,40	24,000	0,2663

Gemäß der Rothamsteder Formel sollte $\frac{1}{y}-\frac{1}{y_1}$ konstant sein, was annähernd zutrifft. $\frac{y}{y_1}$ ist offensichtlich nicht konstant.

Die Formel hat sich auch als brauchbar erwiesen bei der Errechnung von Feldversuchsergebnissen, wie aus Übersicht 8 und 9 hervorgeht.

Neue Stickstoff-Düngemittel.

In den letzten Jahren sind bei uns 3 neue Stickstoffdüngemittel in Versuchen geprüft worden: Kalkstickstoff, Harnstoff und salzsaures Ammoniak. Kalkstickstoff hat sich zu Gerste als gleichwertig mit schwefelsaurem Ammoniak erwiesen, in feuchteren Lagen auch für Zuckerrüben, jedoch nicht für Kartoffeln. Wahrscheinlich spielt der Kalkbedarf der betreffenden Pflanzen in diesen Fällen eine Rolle. Harnstoff erwies sich als gleichwertig in der Wirkung mit schwefelsaurem Ammoniak. Salzsaures Ammoniak erwies sich als weniger wirkungsvoll bei Kartoffeln, zeigte indessen Überlegenheit bei Halmfrüchten. So brachte es vor allem bei Gerste höhere Kornerträge mit etwas geringerem Stickstoffgehalt.

Infolge der niedrigen Preise für Stickstoffdüngemittel interessiert es besonders zu untersuchen, wie *hohe* N-Gaben sich auswirken. So lange die Erträge im gleichen Verhältnis wie die aufgewandte Düngermenge steigen, lohnt es sich, die N-Gaben zu steigern.

Mit der Massenvermehrung geht anfangs keine wesentliche Verschlechterung der übrigen wertbestimmenden Eigenschaften der Ernte einher. Wird die Wachstumsgrenze aber erreicht, so übt der Überschuß an Stickstoff gewisse nachteilige Wirkungen aus, deren Symptome bei Weizen bereits beschrieben worden sind. In ähnlicher Weise hat N-Überschuß auch bei anderen Pflanzen starkes Dunkelgrünwerden und Kräuselungen der Blätter zur Folge. Diese Pflanzen werden besonders leicht anfällig für Krankheiten, das Ausreifen wird verzögert, es bildet sich Lager.

Übersicht 8. *Rothamsted* 1926. *Tatsächlich geerntete Menge im Vergleich zur errechneten Menge Kartoffeln* (*dz/ha*).
(*Balmukand*[1].)

N dz/ha als schwefelsaures Ammoniak	—		1,25		2,5		5,0	
K_2O kg/ha als schwefelsaures Kali	geerntet	berechnet	geerntet	berechnet	geerntet	berechnet	geerntet	berechnet
—	196	178	194	210	236	238	239	259
0,62	196	196	226	234	265	258	280	285
1,24	201	200	230	240	259	264	292	293
2,48	196	202	226	243	262	268	310	298

Mittlerer Fehler des Einzelertrages: 13,1 kg/ha. — Berechnet nach der Formel $\frac{1}{y} = C + \frac{a_k}{k + K} + \frac{a_n}{n + N}$,

worin: y = Kartoffelertrag in dz/ha,
$C = 0{,}00259$,
$K = 0{,}298 \pm 0{,}373$ dz/ha K_2O,
$n = 0{,}455 \pm 0{,}022$ dz/ha N,
$a_k = 0{,}00022 \pm 0{,}00032$ dz K_2O auf den Doppelzentner Kartoffeln.
$a_n = 0{,}00103 \pm 0{,}00064$ dz N auf den Doppelzentner Kartoffeln.

Übersicht 9. *Rothamsted* 1929. *Tatsächlich geerntete Menge im Vergleich zur errechneten Menge Kartoffeln* (*dz/ha*).
(*Kalamkar*[2].)

N kg/ha als schwefelsaures Ammoniak	− P_2O_5						+ P_2O_5					
	—		0,38		0,75		—		0,38		0,75	
K_2O kg/ha als schwefelsaures Kali	geerntet	berechnet	geerntet	berechnet	geerntet	berechnet	geerntet	berechnet	geerntet	berechnet	geerntet	berechnet
—	114	112	128	127	135	135	120	122	139	142	149	150
0,63	122	116	132	133	136	141	126	127	148	146	158	157
1,25	116	116	133	133	140	142	123	127	145	147	162	158

Mittlerer Fehler des Einzelertrages: 2,5 dz/ha. Berechnet nach der Formel:

$$\frac{1}{y} = C + P + \frac{a_k}{k + K} + \frac{a_n}{n + N},$$

worin: y = Kartoffelertrag in dz/ha,
$C = 0{,}00583$,
$P = 0$ in der Serie ohne P_2O_5,
$= -0{,}00072$ in der Serie $+ P_2O_5$,
$a_k = 0{,}000035 \pm 0{,}000298$ dz K_2O auf 1 dz Kartoffeln,
$k = 0{,}0979 \pm 0{,}720$ dz/ha K_2O,
$a_n = 0{,}00168 \pm 0{,}00124$ dz N auf 1 dz Kartoffeln,
$n = 0{,}607 \pm 0{,}030$ dz/ha N.

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[1] *Balmukand*, J. Agricult. Sci. **18**, 602 (1928).

[2] *Kalamkar*, *R. J.*, J. Agricult. Sci. **20**, 440 (1930).

Der relative N-Gehalt des Kornes wird durch steigende Stickstoffgaben nur wenig beeinflußt, solange gleich großen Düngermengen gleich große Ertragszuwachse entsprechen. Beispielsweise bei Gerste: der relative N-Gehalt des Kornes betrug:

	- N	+ 1N	+ 2N
— P	1,92	2,04	2,22
+ P	1,94	2,06	2,13

Dieser Überschuß an Stickstoff bringt all die anderen Bestandteile der Pflanze aus der Reihe, sie nehmen automatisch ab. Die Kohlehydrate trifft es am stärksten. Bei Zuckerrüben und Gerste wird durch zu hohe N-Gaben der Wert des Ernteproduktes ganz erheblich vermindert.

Übersicht 10. *Zusammensetzung der Ernte unter dem Einfluß an N-Düngung.*

		- N	+ 1 N	+ 2 N
Zuckerrüben: Zucker-Prozent		17,98	17,64	17,25
Gerste: Malzextrakt-Prozent	— P	95,60	95,30	93,90
	+ P	95,90	94,60	94,40
Diastasewirkung	— P	54,50	60,00	69,00
	+ P	63,00	68,00	68,50
Futterrüben: N-Prozent im Rübenfrischgew. .		0,124	0,130	0,199
In der Trockensubstanz		0,790	0,970	1,610
Zucker-Prozent		11,1	8,9	8,0

Die Erträge betrugen: dz/ha.

	- N	+ 1N	+ 2N
Rübenkörper	226	232	231
Blätter . .	259	290	310

Rothamsted Report **1928**, 147.

Mancher englische Landwirt ist nicht zu bewegen, seiner Gerste eine N-Düngung oder sogar überhaupt eine Düngung angedeihen zu lassen aus Furcht, er könne die Qualität verderben. Auch andere Bestandteile der Pflanze werden vermindert durch Anwendung von Stickstoff, so der Trockensubstanzgehalt des Weizens auf Broadbalk.

Übersicht 11. *Einfluß von Stickstoff auf die Zusammensetzung von Weizen.*

	Korn		Stroh	
	Ungedüngt	96,3 kg/ha N	Ungedüngt	96,3 kg/ha N
	Parz. 3	Parz. 10 A	Parz. 3	Parz. 10 A
N	1,90	2,15	0,50	0,67
Asche . . .	2,01	1,80	6,44	5,32
P_2O_5	1,00	0,85	0,24	0,16
K_2O	0,66	0,60	0,93	0,87
MgO	0,21	0,19	0,13	0,09
CaO	0,057	0,065	0,25	0,32

Es ist unsicher, inwiefern diese Veränderungen die Pflanzen oder den Wert des Produktes beeinflussen. Der Organismus der Pflanze scheint nur innerhalb eines engen Bereiches des Kohlenstoff-Stickstoff-Verhältnisses normal zu gedeihen. Wird dieses zu eng, so krankt die Pflanze. Auch die Tierzelle leidet unter Stickstoff-Überschuß, sie hat jedoch die Fähigkeit, den Überschuß auszuscheiden, was bei der Pflanze nicht der Fall ist.

Die Stickstoffumbildungsprodukte nehmen nicht alle im gleichen Verhältnis zu, wie der Gehalt an Gesamtstickstoff in der Pflanze anwächst. *Bishop* hat diesen Umbildungsprozeß bei Gerste in Rothamsted eingehend untersucht. Im Gerstenkorn nimmt der Gehalt an Hordein in höherem Maße zu als irgendeine andere Verbindung, woraus hervorgeht, daß es am meisten aufgespeichert wird. Der Gluteingehalt nimmt in geringerem Maße zu, und noch weniger werden die salzlöslichen Verbindungen angehäuft. Die Zusammensetzung der verschiedenen Proteine und ihr mengenmäßiges Auftreten im Weizenkorn hängt lediglich von der Gesamtmenge des zur Verfügung stehenden Stickstoffs ab, einerlei, ob dieser in Form von Düngemitteln verabfolgt wird, ob er dem Bodenvorrat entstammt, oder ob er mit den Niederschlägen aus der Atmosphäre in den Boden gelangt. Die einfachsten Beziehungen bestehen für das schon erwähnte Hordein: Bei allen zweizeiligen Gerstensorten, die bisher untersucht worden sind, entspricht der gleichen Menge Gesamtstickstoff die gleiche Menge dieses Proteins (H). Wenn N der prozentuale Stickstoffgehalt der Trockensubstanz ist, so berechnet sich die in der Trockensubstanz von 1000 Körnern enthaltene Menge Hordein als:

$$H = -0{,}089 + 0{,}422N + 0{,}0727N^2.$$

Die restlichen Stickstoffverbindungen — die salzlöslichen und das Glutein — schwanken in ihrem Anteil je nach Sorte. Im vollreifen Korn hängt die Größe dieses Anteils lediglich vom Gesamtstickstoffgehalt und von der Sorte ab. Boden, Witterung und Düngung üben keinerlei Einfluß aus.

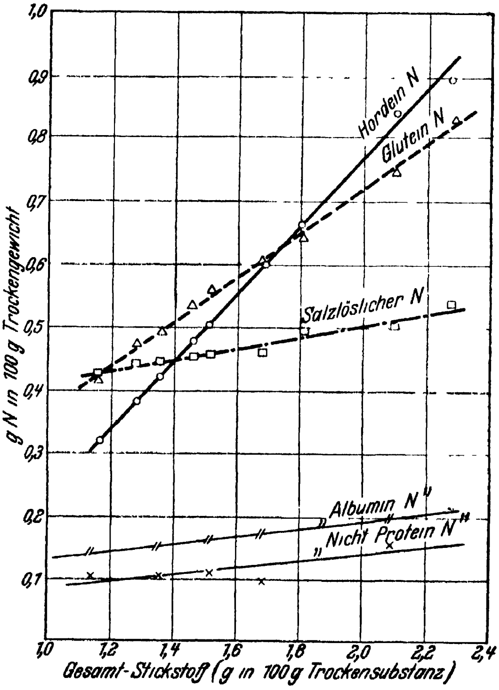

Abb. 2.

Bishop konnte ferner zeigen, wie man aus den Daten für Gesamtstickstoffgehalt des Gerstenkornes, die Menge des daraus herzustellenden Malzes berechnen kann, was für die Malzfabrikanten von außerordentlicher Bedeutung ist. Er hat die sehr schwierige und langwierige Analyse, die früher zu derartigen Untersuchungen nötig war, also wesentlich vereinfacht und verbilligt: Auf einem von ihm konstruierten Rechenschieber kann man den Wert ohne weiteres ablesen. Ein anderes, sehr einfaches Verfahren gestattet, die Diastasewirkung vom Malz vorauszubestimmen, der einer beliebigen Temperatur zur Fermentierung ausgesetzt wird. Auf Grund der guten Übereinstimmung zwischen erwarteten und gefundenen Werten kann die Diastasewirkung als Maßstab für den Mälzprozeß angesehen werden. Die Gleichungen lauten z. B. für die Sorte Plumage Archer:

1. Für Extrakt: $E = 110{,}1 - 11{,}2 \cdot N + 0{,}18 \cdot G$;
2. Für Diastase Wirkung: D.W. $= 29 \cdot N + 0{,}4 \cdot G - 21$;
3. für ständig löslichen Stickstoff: St.l.St. $= 33\,\% \cdot N$,

wobei N = Gesamt-N-Gehalt getrockneter Gerste,
G = Trockengewicht von 1000 Körnern.
D.W. gilt für eine Darrtemperatur von 82,2 °C[1].

Die übrigen Düngesalze.

Kalidüngemittel: Diese stehen in engen Beziehungen mit den Stickstoffdüngemitteln. Bei niedriger N-Zufuhr hat Kali nur wenig Einfluß auf das Pflanzenwachstum, bei reichlicher N-Zufuhr dagegen ist die Wirkung recht erheblich. Die Wirkung von Kali besteht darin, die Leistungsfähigkeit des Blattes bei der Assimilation von Kohlensäure zu steigern. Es vergrößert allerdings die Blattfläche nicht. Daraus ergibt sich, daß Kali eine Gegenwirkung gegen die schädlichen Folgen von Stickstoffüberschuß ausübt. Dies zeigt sich besonders deutlich bei den Rothamsteder Versuchen mit Futterrüben. Auf den Kali-Mangel-Parzellen bilden die Rübenblätter nur wenig Rübenkörper. Bei Verabreichung von Kali wird die Blattentwicklung kaum gefördert, die Pflanze kann dagegen den Stickstoff besser ausnutzen und infolgedessen auf die gleiche Blattmasse mehr Rübenmasse erzeugen. Typische Ergebnisse bringt folgende Zusammenstellung:

Die Ergebnisse sind graphisch dargestellt in Abb. 3 und 4.

Die Pflanzen mit Stickstoffüberschuß bei Fehlen von Kali in der Düngung bleiben nicht nur klein, sie sind anormal in jeder Beziehung und sind sehr anfällig für Krankheiten. Die Blätter sind tiefgrün,

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[1] Nähere Einzelheiten siehe a) Proteine: J. Inst. Brewing **34**, 101 (1928); **35**, 316 u. 323 (1929); **36**, 336 (1929). b) Berechnungsmethoden: J. Inst. Brewing **36**, 421 (1930). Die Veröffentlichungen über ständig löslichen Stickstoff und Diastasewirkung sind in Vorbereitung.

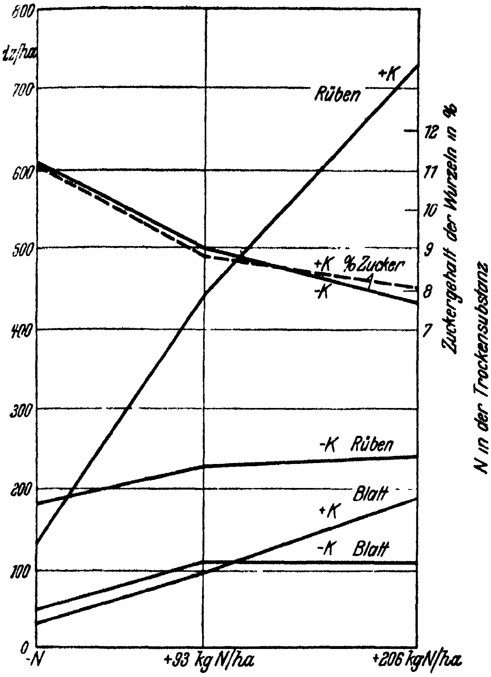

Abb. 3

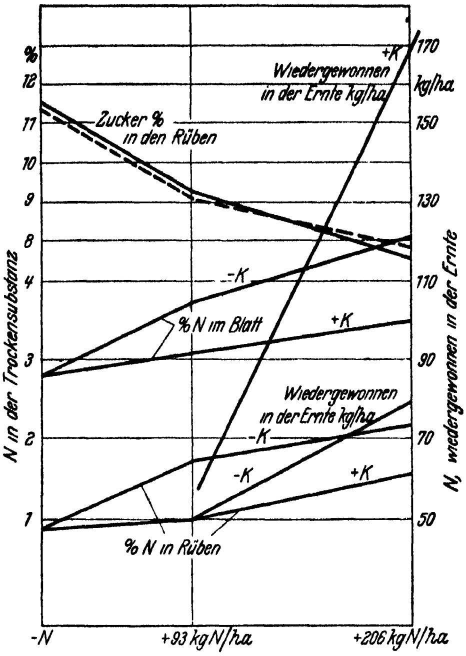

Abb. 4.

Übersicht 12. *Einfluß von Kalidüngung auf Menge und Zusammensetzung der Ernte von Futterrüben. 1902.*

	— N		+ 96,3 kg/ha N		+ 206 kg/ha N	
	— K	+ K	— K	+ K	— K	+ K
	Parz. 5 — O	Parz. 6 — O	Parz. 5 — O	Parz. 6 — O	Parz. 5 — O	Parz. 6 — O
Ertrag: dz/ha:						
Blätter	47,0	30,1	105,1	92,6	107,0	187,7
Rüben	182,4	128,1	224,6	441,1	239,9	742,0
Zucker	20,4	14,2	20,2	39,3	18,5	59,4
Zucker-Prozent	11,2	11,1	9,0	8,9	7,7	8,0
N-Prozent in:						
Blätter[1]	2,63	2,67	3,67	3,04	4,65	3,51
Rüben[1]	0,87	0,79	1,69	0,97	2,24	1,61
Wiedergewinnung des aufgewandten N.						
N-dz/ha in:						
Blätter	0,113	0,085	0,350	0,221	0,500	0,527
Rüben	0,245	0,159	0,513	0,574	0,668	1,470
Gesamt:	0,358	0,244	0,863	0,795	1,168	1,997
— N der Düngung . .	—	—	0,358	0,244	0,358	0,244
Wiedergewonnener N: dz/ha			0,505	0,551	0,810	1,753
Desgl.: Prozent der aufgew. Menge . . .			54%	59%	40%	85%

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[1] Getrocknet bei 100°.

gewellt und stehen dicht gedrängt nebeneinander, anstatt sich zum Lichte hin auszubreiten. Sie sind sehr anfällig für Uromyces betae und andere Pilz- und Bakterienkrankheiten, werden leicht schlaff und sterben vorzeitig ab. Es entstehen somit zahlreiche Lücken auf dem Felde. Das ist die Folge eines zu engen C : N-Verhältnisses. Bei Verabreichung von Kalisalzen wird mehr Kohlensäure assimiliert und das C : N-Verhältnis ausgeglichen. Der prozentuale N-Gehalt des Blattes wird herabgedrückt von 4,6 auf 3,5. Es ist freilich bemerkenswert, wie wenig die Zusammensetzung der Rüben dadurch beeinflußt wird: Der relative Zuckergehalt nimmt mit zunehmendem N-Gehalt etwas ab, aber der Einfluß des Kalis ist nur schwach. Das Blatt scheint demnach ein Gemisch von ähnlicher Zusammensetzung zur Wurzel hinabzusenden, ob mit Kali gedüngt ist oder nicht. Bei genügender Kalizufuhr kann es aber größere Mengen ableiten als im Falle von Kaliknappheit.

Davis zeigte in unseren Laboratorien, daß Rohrzucker das 1. Produkt der Photosynthese im Blatt der Futterrübe ist. Dieser wird in den Blattadern und im Stengel umgebildet zu Hexosen. In dieser Form wird das Assimilationsprodukt beweglicher und wandert in die Wurzel. Dort wird es rückverwandelt in Rohrzucker und wird dadurch wieder weniger gut transportabel.

Die enge Beziehung zwischen Stickstoffdüngemitteln und Kalidüngemitteln zeigt sich auch in den Versuchen mit Kartoffeln: Bei Fehlen von Stickstoff haben die Kalidüngemittel keinerlei Wirkung, bei Fehlen von Kalidüngesalzen bewirkt umgekehrt Stickstoffdüngung nur wenig Ertragssteigerung. Im letzteren Falle treten dann Anormalitäten auf, die denen bei Rüben ähneln. Die Blätter färben sich tief dunkelgrün und haben ungesundes Aussehen. Die Ertragsverhältnisse dieser Versuche mit Kartoffeln sind in den Übers. 3 und 8 wiedergegeben worden. Die Einwirkung der Düngung auf die Zusammensetzung der Kartoffeln geht aus Übers. 13 hervor, in der Ergebnisse von Rothamsteder Versuchen aus dem Jahre 1930 dargestellt sind.

Stickstoffdüngemittel erhöhen den relativen Stickstoffgehalt der Knolle, Kalidüngemittel vermindern ihn. Unter den verschiedenen Kalidüngesalzen hat das schwefelsaure Kalium keinen Einfluß auf den relativen Trockensubstanzgehalt, während Düngesalze, in denen Kaliumchlorid enthalten ist, ihn vermindern. Namentlich die stickstofffreien Substanzen werden davon betroffen. Mancherlei Beispiele könnten angeführt werden für die Erhöhung der Widerstandsfähigkeit gegen Krankheiten durch Kalidüngesalze. So wird in Woburn die Gerste bei Kalimangel von Fusarium culmorum befallen, im Lea-Tal leiden die Tomaten unter verschiedenen Krankheiten. Der Grad der Anfälligkeit wird durch Kaligaben herabgesetzt.

Übersicht 13. *Rothamsted 1930. Der Einfluß von Düngemitteln auf die Zusammensetzung von Kartoffeln.*

Düngung dz/ha	Trockensubstanz %	Stickstoff in frischen Knollen %	Stickstoff in Trockensubstanz %
	1. *Schwefelsaures Ammoniak.*		
—	22,8	0,305	1,34
0,25 N	23,0	0,322	1,40
0,50 N	23,0	0,337	1,47
Mittlerer Fehler	0,1	0,0028	—
	2. *Kalidüngesalze* (Mittel).		
—	23,1	0,329	1,42
0,5 K_2O	23,0	0,319	1,38
1,0 K_2O	22,7	0,317	1,39
Mittlerer Fehler	0,1	0,0028	—

3. *Kalidüngesalze* (einzelne Salze).

K_2O	0,5	1,0	0,5	1,0	0,5	1,0
Als schwefelsaures Kali	23,2	23,3	0,316	0,334	1,36	1,43
„ salzsaures Kali	23,0	22,7	0,316	0,310	1,37	1,36
„ 30proz. Kalidungesalz	22,8	22,1	0,324	0,307	1,47	1,39
Mittlerer Fehler	0,17		0,0049		—	

Übersicht 14. *Kali-Gaben und Widerstandsfähigkeit gegen Krankheiten.*

Feldfrucht	Krankheit	Grad der Anfälligkeit + K_2O	Grad der Anfälligkeit − K_2O
Gerste (Woburn)	Fusarium culmorum	1	1,6
Tomate (Lea Valley)	Strichelkrankheit	1	2—2,5
	Fleckigkeit der Früchte	1	5

Wallace, Long Ashton, hat gezeigt, daß der prozentuale Kaligehalt der Trockensubstanz von Stachelbeer- und Johannisbeersträuchern in Beziehung steht zu ihrem Gesundheitszustand.

Übersicht 15. *Kali-Gehalt der Trockensubstanz von Stachel- und Johannisbeersträuchern und seine Beziehung zu ihrem Gesundheitszustand.*

	Tonböden gesund	Tonböden leicht erkrankt	Sandböden teilweise Fehlernte	Sandböden völliger Fehlschlag
Asche in der Trockensubstanz	12,55	9,25	8,58	8,90
K_2O in der Asche	23,11	17,92	6,63	3,86

Kalidüngesalze erleichtern offenbar auch die Wasseraufnahme der Pflanzen. Das zeigt sich in Rothamsted bei Kartoffeln deutlich in trockenen Frühjahren. Unter den Parzellen des Wiesendüngungsversuches neigen die besonders zu Lager, die nicht mit Kali gedüngt sind. Die Turgeszenz ist bei den Gräsern dieser Bestände schwächer.

Besonders lohnen Leguminosen eine Kalidüngung durch vermehrtes Wachstum und Erhöhung der Winterfestigkeit. Das dürfte mit dem Umstand zusammenhängen, daß die Knöllchenbakterien in den Wurzeln bezüglich ihrer Versorgung mit Kohlehydraten von der Pflanze abhängen. Die Leguminosen speichern Reservestoffe für den Winter in ihren Wurzeln auf, und je mehr Kohlehydrate in diese hineinwandern, um so größer ist für die Pflanzen die Aussicht, gut durch den Winter zu kommen. Einige der Rothamsteder Daten bringt Übersicht 16:

Übersicht 16. *Einfluß von Kaligaben auf die Klee-Heu-Ernten.* (Rotham. Rept. 1924: 114.)

	1922		1923	1924	
	Heu dz/ha	Trockensubstanz dz/ha	Heu dz/ha	Heu dz/ha	Trocken substanz dz/ha
Volldüngung	29,3	20,9	47,3	90,8	70,2
Ohne Kali	20.7	15.5	29.8	72.8	57.0

In beiden Fällen wurden die Düngemittel, wie allgemein üblich in England, zur Gerste gegeben, in die der Klee eingesät wird.

Die Gerstenerträge betrugen:

	1922		1923	
	Korn dz/ha	Stroh dz/ha	Korn dz/ha	Stroh dz/ha
Volldüngung	20,15	21,5	22,12	24,9
Ohne Kali	16.92	17.7	21.29	21.9

In einem unserer Versuche war der Klee ausnahmsweise 2 Jahre stehengeblieben, sonst pflügen wir ihn nach 1 Jahre um. Die Gerstenüberfrucht stand sehr dünn, und die Kalisulfatgabe hatte im 1. Jahre wenig genützt. Sie befähigte die Pflanzen aber, den darauffolgenden Winter besser zu überstehen:

	1. Jahr (1921) dz/ha	2. Jahr (1922) dz/ha
Mit Kali . .	53,82	25,06
Ohne Kali .	59,28	17,14

Kaligaben beeinflussen auch den Reifeprozeß des Kornes, und zwar erhöhen sie das Hektolitergewicht. Andererseits drücken sie, wie wir sahen, den Stickstoffgehalt bei Gerste und verbessern auf diese Weise die Brauqualität.

Die Wirkung von Kalidüngesalzen auf die Zusammensetzung der Ernte wird beeinflußt von 2 Faktoren: 1. Kalisalze sind leicht aufnehmbar für die Pflanze und vermindern die Aufnahmefähigkeit für andere mineralische Substanzen. 2. In Gegenwart von genügenden Mengen Stickstoff steigern sie die Kohlensäureassimilation und vermindern unter Umständen den Stickstoffgehalt des Ernteprodukts.

Natronsalze: Natronsalze haben ganz entschieden einen eigenen Düngewert. 1. Sie erleichtern die Aufnahme für Kali aus dem Boden. 2. Sie sind von unmittelbarer Bedeutung für Futter- und Zuckerrüben, bei denen sie in ihrer Wirkung offenbar verschieden sind von Kalisalzen.

Ein Beispiel für die Beeinflussung der Kaliaufnahme durch Natronsalze bietet der Weizenversuch auf Broadbalkfield:

Übersicht 17. *Broadbalkfield. Durchschnittlicher jährlicher Kaligehalt der Weizenernten* (kg/ha).

Parz. Nr.	Düngung	1852—1861		1862—1871		Korn + Stroh 1852—1871	Der Dungung entstammendes K_2O
		Korn	Stroh	Korn	Stroh		
13	N + P + K .	12,6	47,0	13,7	48,2	60,8	29,0
12	N + P + Na .	12,8	38,1	12,8	29,6	46,6	14,8
11	N + P	10,4	24,2	9,7	19,3	31,8	—

Sehr deutlich ist auch die Wirkung von Kaligaben bei Zuckerrüben. Hier sind die Kalidüngesalze in unseren Versuchen dem Kaliumchlorid überlegen.

Übersicht 18. *Zuckerrubenerträge* (dz/ha).

	Woburn 1926	Colchester 1929		Rothamsted 1929	
			Zucker %		Zucker %
Grunddüngung . . .	376	149	17,64	192	18,38
+ Kaliumchlorid[1] . . .	376	163	17,63	185	18,39
+ Kalidüngesalz . . .	403	191	18,00	196[2]	18,39
+ Kochsalz	—	173	17,84	189	18,35

Eine Wirkung des Kochsalzes dürfte darin bestehen, die Konzentration des Gewebesaftes im Blatt zu erhöhen und dadurch den schwierigen Vorgang zu erleichtern, Zucker stielabwärts in das Wurzelgewebe hineinzubefördern, das mit stärker konzentriertem Zellsaft erfüllt ist.

Phosphate: Am auffälligsten sind die Wirkungen der Phosphate auf die Ausbildung des Wurzelsystems, die Bestockung bei Getreide und die Beschleunigung des Reifeprozesses.

Der Einfluß auf die Ausbildung des Wurzelsystems ist sehr stark bei Brassicarüben, und daher ist es auch allgemein üblich in England, Wasserrüben und Kohlrüben eine Gabe von 6—7 dz/ha Superphosphat angedeihen zu lassen, um ihre Jugendentwicklung zu beschleunigen und sie eher hacken zu können. Einige der englischen Böden sind sehr arm an Phosphorsäure und lohnen eine Düngung recht gut.

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[1] 2 dz/ha.

[2] 2 dz/ha KCl + entsprechende Menge NaCl.

Der Einfluß auf die Bestockung der Getreidearten ist sehr groß. In späteren Entwicklungsstadien bewirken die Phosphate eine Beschleunigung des Reifeprozesses der Pflanze und üben damit eine dem Wassermangel ähnliche, aber viel gemäßigtere Wirkung aus. Aus diesem Grunde werden sie in einigen Gegenden Nordenglands zu Weizen, in Westengland zu Hafer angewandt. Die Ernte wird dadurch um einige Tage verfrüht und die Gefahr von Verlusten durch ungünstige Witterung vermindert. Die Anbaugrenze könnte auf diese Weise für verschiedene Feldfrüchte nach dem Norden zu verschoben werden. Die Beschleunigung des Reifeprozesses ist sehr schön bei den Gerstenparzellen Hoosfields zu sehen: Teilstücke mit Phosphatdüngung leuchten zu einer Zeit schon goldgelb, wenn die anderen noch grün sind.

Außerdem üben die Phosphate noch indirekt verschiedene Wirkungen aus: Die Gerste schoßt einige Tage früher als bei Mangel an Phosphorsäure und entgeht daher eher den Blumenfliegenschäden (Chlorops taeniopus, Meig.). Die Larven dieser Fliege wandern vom obersten Blatt her, wo die Fliege ihre Eier abzulegen pflegt, abwärts bis sie die Ährenanlage erreicht haben.

Durch Phosphatdüngung werden also die Ernten sicherer, besonders die von Kohlrüben, die in Jahren mit ungünstiger Witterung viel mehr auf die Beine gebracht werden, als sie von einer Phosphatgabe unter günstigen Witterungsverhältnissen Vorteil haben. Durch Anwendung dieser Düngemittel werden demnach die extremen Wirkungen schlechter und guter Jahre etwas ausgeglichen. Nachfolgende Übersicht zeigt dies.

Phosphatwirkung bei Kohlrüben.
(Rotham. Rept. 1925: 18.)

	Ungünst. Witterung (1920) dz/ha	Günstige Witterung (1924) dz/ha
Ungedüngt	82,9	434,6
Volldüngung	409,5	517,5
Ohne Phosphorsäure	233,6	479,8
Ertragszuwachs durch Phosphat	175.8	37.7

Physiologische Untersuchungen lehren, daß Phosphate enge Beziehungen zum Wachstumsvorgang und zur Atmung haben: 1. Phosphorsäure ist ein Bestandteil des Zellkernes und spielt als solcher eine wesentliche Rolle bei der Zellteilung und bei der Entwicklung der Meristemgewebe. 2. Sie ist nötig zum normalen Verlauf der Umwandlung der Kohlehydrate und für die Tätigkeit der Chloroplasten.

Die Oxydation des Zuckers in der lebenden Zelle verläuft wahrscheinlich unter gleichzeitiger Bildung eines Hexose-Phosphates. *Harden* und *Young*[1] fanden, daß diese Verbindung bei der Vergärung von Zucker durch Hefe auftritt, und *Otto Meyerhoff*[2] wies nach, daß das gleiche für die Bildung von CO_2 in den Muskelzellen und ganz allgemein für die Veratmung von Kohlehydraten in lebenden Zellen gilt.

Eine ganz besondere Wirkung üben die Phosphate auf die Leguminosen in Grasbeständen aus. Viele der Weiden auf den Tonböden Englands haben sehr dürftigen Grasbestand mit wenig Klee darin und zeigen viele Kahlstellen. Eine Düngung von 7—12 dz/ha Thomasmehl ruft eine ganz wunderbare Wandlung hervor: Wilder Weißklee sprießt plötzlich hervor und füllt die Lücken. Der Wert des Landes zu Weidezwecken wird ganz erheblich verbessert. Die Erklärung dafür scheint in dem Umstande zu liegen, daß die Phosphate die Metamorphose der Knöllchenbakterien vom nichtbeweglichen Stadium zur beweglichen Form beschleunigen und dadurch die Infektion der Wurzeln erleichtern. Dies hat wiederum zur Folge, daß der Proteingehalt der Weidepflanzen erhöht wird (vgl. Übersicht 4).

Die Pflanzen decken ihren Bedarf an Phosphorsäure innerhalb der ersten Wochen ihres Wachstums. So kommt es, daß die Phosphatwirkungen besonders intensiv hervortreten, wenn die Düngung in wasserlöslicher Form verabreicht wird. Dies ist besonders der Fall in Gegenden mit geringen Niederschlagsmengen. In feuchteren Gebieten sind dagegen die schwerer löslichen Formen vorteilhafter.

Übersicht 19. *Wiedergewinn an Düngerphosphorsaure bei verschiedenen Feldfrüchten* (kg/ha).

	Ort der Versuchsanstellung	Düngung P_2O_5	P_2O_5-Gehalt der Ernte			Wiedergewonnen in der Ernte %
			$-P_2O_5$	$+P_2O_5$	Differenz	
	Unter normalen Verhältnissen.					
Superphosphat:						
Kohlrüben . .	Roth.: Little Hoos.	78,4	—	—	11,2 (7,8)	24
Heu: 1. Jahr .	Essex	126,0	29,1	42,6	13,5	11
1. Jahr .	—	126,0	—	—	—	6
Thomasmehl:						
Heu: 1. Jahr .	—	112,0	11,4	16,1	5,1	3
1. 4 Jahre	—	—	26,1	42,6	16,6	15
	Bei Phosphorsäuremangel.					
Superphosphat:						
Heu	Roth. Park-Grass	72	11,2	29,1	18,0	25
Gerste	Roth. Hoosfield	72	11,7	25,3	13,5	19
Weizen	Roth. Broadbalkfield	72	16,2	26,7	10,8	14

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[1] Vgl. *A. Harden,* Alcoholic fermentation. Longman u. Co. 1923.

[2] Chemical Dynamics of life phenomena. Philadelphia 1924.

Es erhebt sich die Frage: Wieviel von der mit der Düngung verabfolgten Phosphorsäure wird von der Pflanze aufgenommen? Die wenigen Versuche, die in dieser Beziehung gemacht worden sind, sprechen für keine besonders hohe Ausnutzung der Düngerphosphorsäure. Unter normalen Bedingungen wurden, selbst wenn eine ausreichende Beidüngung von Stickstoff und Kali erfolgte, nur verhältnismäßig kleine Mengen in der Ernte wiedergewonnen:

Die reifebeschleunigende Wirkung der Phosphorsäure ist nachteilig für leichte Böden. Sie übt in dieser Beziehung die entgegengesetzte Wirkung von Kalidüngesalzen aus. Dies zeigen die in Übersicht 20 zusammengestellten Ertragsverhältnisse bei Zuckerrüben aus dem trockenen Jahre 1929. Der Versuch bestand aus einer großen Zahl von Teilstücken und war mit Wiederholung angelegt worden:

Übersicht 20. *Phosphatwirkung bei Zuckerrüben in einem trockenen Jahre.*

Düngung	Schwerer Boden (Rothamsted)		Leichter Boden (Woburn)	
	− Superphosphat	+ Superphosphat	− Superphosphat	+ Superphosphat
Grunddüngung[1]	174	192	207	215
Kaliumchlorid	186	185	216	190
Kalidüngesalz	182[2]	196[2]	228	208
Mittlerer Fehler	2.8		10.6	

Was die Beeinflussung der chemischen Zusammensetzung der Ernte durch Phosphate betrifft, so läßt sich allgemein feststellen, daß die Beziehung zwischen verabfolgter Menge und von der Pflanze aufgenommener Menge logarithmischer Art ist. Kleine Phosphorsäuregaben, auf einem Boden angewandt, der arm an P_2O_5 ist, vermehren die Aufnahme dieses Nährstoffes durch die Pflanze ganz erheblich. Steigende Gaben können unter günstigen Umweltverhältnissen proportionale Steigerung der Wachstumstätigkeit zur Folge haben. Sind die übrigen Bedingungen nur schlecht erfüllt, so wird das Wachstum verhältnismäßig weniger gefördert und infolgedessen überschüssige P_2O_5 im Pflanzengewebe angehäuft. So war beispielsweise:

Der P_2O_5-Gehalt von Gras

	in P_2O_5-armem Boden		bei mittlerem P_2O_5-Gehalt	
	− Superphosphat %	+ Superphosphat %	− Superphosphat %	+ Superphosphat %
P_2O_5-Gehalt . .	0,29	0,71	0,96	0,93
CaO-Gehalt . .	0,59	0,94	1,16	1,16

Phosphor ist aber ein lebenswichtiger Bestandteil bei der Tierernährung und aus den zahlreichen Versuchen geht hervor, daß Grünfutter mit weniger als 0,1 — 0,2 % P_2O_5 in der Trockensubstanz dem tierischen Organismus nicht genug Phosphor zuführt und zu krankhaften Mangelerscheinungen Veranlassung gibt. Besonders unangenehm tritt dies in Südafrika in Erscheinung, aber auch aus Australien und anderen Ländern wird derartiges berichtet.

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[1] Ohne Kali und Natronsalze.

[2] 2 dz/ha Kaliumchlorid und entsprechende Kochsalzmenge.

Prozente P_2O_5 in der Trockensubstanz von			
nahrhaftem Gras: keine krankhaften Mangelerscheinungen bei den Tieren		nichtnahrhaftem Gras: krankhafte Mangelerscheinungen bei den Tieren	
Romney Marsch	0,60	Transvaal	0,10
England: Mittel	0,73	Bechuanaland	0,04
		Victoris	0.10

(Nach *J. B. Orr*, Minerals in Pasture, London 1929).

Abhilfe kann in solchen Fällen nur schaffen, entweder das Land mit Phosphaten zu düngen, oder Knochenmehl an die Tiere zu verfüttern.

Die Wirkung von Stallmist.

Die alten Rothamsteder Versuche zeigten, daß die damals gerade erst entdeckten künstlichen Düngemittel etwa die gleiche Wirkung auf das Wachstum der Pflanzen ausüben wie Stallmist. Im Laufe der Zeit wurde dies weiterhin bestätigt, es stellten sich aber auch verschiedene bedeutsame Unterschiede heraus.

1. Die Wirkung von Stallmist hält eine Reihe von Jahren hindurch im Boden vor. Diese Nachwirkung tritt besonders deutlich zutage, wenn der betreffende Boden längere Zeit Jahr für Jahr eine Stallmistdüngung erhalten hat. Auf Hoosfield erhielt z. B. eine Parzelle 20 Jahre lang (1852—1871) Stallmist. Seitdem blieb sie ungedüngt und weist doch auch heute noch einen beträchtlich höheren Ertrag auf als die ungedüngte Vergleichsfläche (vgl. Übersicht 21 und Abb. 5). Eine einmalige Gabe, wie sie in der Praxis verabfolgt wird, hält natürlich nicht so lange vor, ihre Nachwirkung kann aber immerhin zum mindesten noch nach 4 Jahren nachgewiesen werden. Der Unterschied zwischen fettem

Übersicht 21. *Rothamsted-Hoosfield. Ununterbrochener Anbau von Gerste. Durchschnittlicher Jahresertrag an gereinigtem Korn* (Hektol./ha).

	20 Jahre 1852 bis 1871	5 Jahre 1872 bis 1876	5 Jahre 1877 bis 1881	10 Jahre 1882 bis 1891	10 Jahre 1892 bis 1901	10 Jahre 1902 bis 1911	10 Jahre 1913 bis 1922	8 Jahre 1923 bis 1930
Stallmist jedes Jahr	—	44,64	45,72	42,84	39,87	39,87	35,28	24,30
Stallmist jedes Jahr von 1852 bis 1871, danach unged.	43,47	35,19	26,28	23,85	18,27	16,47	18,90	9,09
Ungedüngt	19.80	12.15	12.96	14.22	9.36	8.73	12.87	4.77

Stallmist, wie er anfällt, wenn das Vieh mit Kraftfuttermitteln gefüttert wird, und magerem ist nicht sehr groß. Er macht nur einen Unterschied in der Nachwirkung von etwa 1 Jahre aus. Im 2. und gar erst im 3. Jahre ist der Unterschied jedenfalls verwischt. Andere Stickstoffformen wie schwefelsaures Ammoniak, Natronsalpeter und auch Rapskuchen haben keine derartig dauerhafte Wirkung. Der Stickstoff wirkt vielmehr nur im Jahre der Anwendung. Phosphorsäure und Kalidüngemittel wirken dagegen nach. Allem Anschein nach ist es das Stroh, das die dauerhafte Wirksamkeit des Stickstoffes und andere Besonderheiten verursacht. Denn das Stroh ist ja die Quelle für den Humus. Die dauerhafte Wirkung des Stallmistes war den englischen Gesetzgebern bekannt, daher setzten sie fest, daß ein Landwirt bei Aufgabe seiner Pachtwirtschaft Entschädigung zu erhalten hat für Stallmistdüngungen, die er 3—4 Jahre vor Verlassen seiner Pachtung angewandt hat.

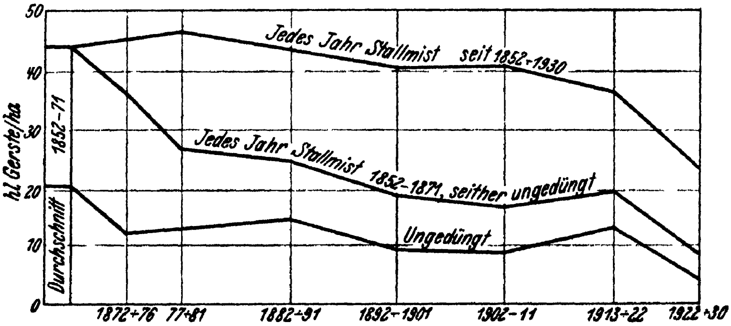

Abb. 5.

2. Künstliche Düngemittel erhalten die Bodenfruchtbarkeit nicht so gut aufrecht wie Stallmist. Der Unterschied ist freilich nicht sehr groß und er tritt erst nach langen Jahren und bei intensiver Bewirtschaftung in Erscheinung. Das rigoroseste Verfahren ist unausgesetzter Anbau von Getreide.

Sowohl in Rothamsted wie auch in Woburn sind die Erträge selbst bei Anwendung starker Volldüngungsgaben mehr zurückgegangen als auf den Stallmistparzellen.

3. Mit künstlichen Düngemitteln erzielt man gewöhnlich keine so sicheren Erträge wie mit Stallmist. In günstigen Jahren mögen sie besser sein, in ungünstigen sind sie dafür um so schlechter. Mit anderen Worten, die jährlichen Ertragsschwankungen sind bei Verwendung künstlicher Düngemittel größer als bei Stallmistdüngung.

4. Auf Klee und verschiedene andere Pflanzen, namentlich einige Fruchtbäume, scheint Stallmist eine günstige Wirkung auszuüben, die bisher noch mit keinem künstlichen Düngemittel erzielt werden konnte.

Zur Zeit ist es noch nicht möglich, diese Unterschiede restlos zu erklären. Wie es scheint, hängen sie zusammen mit der Verrottung des Strohs, der Quelle für den Bodenhumus. Drei Eigenschaften des Stallmistes sind indessen eingehender untersucht worden:

a) Stallmist reichert den Boden mit Humus an und erhöht auf diese Weise dessen wasserhaltende Kraft, was künstliche Düngemittel nicht vermögen. Der Unterschied wird besonders deutlich, wenn die unterschiedliche Düngeweise eine Reihe von Jahren gleichmäßig beibehalten wird, und zwar zeigt er sich am besten bei den empfindlicheren Gewächsen — wie Futterrüben —, die große Mengen von Wasser während der Vegetationszeit verbrauchen. In trockenen Jahren pflegen sich die Futterrüben auf den Stallmistparzellen Barnfields schon zu einer Zeit kräftig zu entwickeln, zu der auf den Flächen mit künstlicher Düngung kaum die Keimung vollzogen ist. Später verwischen sich die Unterschiede oft, wenn das Jahr genügend Niederschläge bringt, sie können aber auch selbst dann noch am Ertrag deutlich hervortreten.

b) Im Stallmist sind alle Nährstoffe enthalten, die für das Pflanzenwachstum erforderlich sind, wogegen mit den üblichen Handelsdüngemitteln fast ausschließlich Stickstoff, Phosphorsäure und Kali verabfolgt werden. Jetzt wissen wir indessen, daß die Pflanzen zu ihrer Ernährung noch andere Elemente benötigen. Solange diese fehlen, haben sie Schwierigkeiten im Fortkommen.

So spielt die Kalkfrage hier hinein. Mangel an Calcium verursacht Mißbildungen des Wurzelsystems und ist wahrscheinlich einer der Gründe für die schädliche Wirkung saurer Böden.

Auch der Eisengehalt des Stallmistes könnte eine Rolle spielen. Eisenmangel hat in Neuseeland Veranlassung gegeben zu einer Erkrankung von Haustieren. Besonders Rindvieh wird hiervon betroffen, weniger Schafe. Dem kann abgeholfen werden durch eine Düngung der Weiden mit Eisensulfat. Einfacher als dieses Verfahren ist freilich eine direkte Beimischung eines Eisensalzes zur Futterration.

Dem Mangel an Mangan schiebt man heutzutage große Bedeutung zu. Man glaubt, daß dadurch die Dörrfleckenkrankheit des Hafers verursacht werde.

Auch Sulfate sind wichtig für die Pflanze, und unter Umständen kann ein sonst sehr wertvolles Düngemittel auf Grund dessen, daß es kein Sulfat enthält, weniger wirksam sein als ein sulfathaltiges.

Chloride, besonders Ammoniumchlorid, haben in Rothamsted eine wichtige Bedeutung für Gerste. Salzsaures Ammoniak gibt jedenfalls immer etwas höhere Erträge mit niedrigerem Stickstoffgehalt als schwefelsaures — Jodide haben sich andererseits als einflußlos erwiesen. Natronsilicat hat auf den Gerstenparzellen zur Folge gehabt, daß der Verbrauch an Phosphorsäure haushälterischer gestaltet wurde. Die

Wirkung dieses Düngemittels scheint sich aber zum Teil auf Veränderungen im Boden zu erstrecken. Auf den betreffenden Teilstücken des Wiesendüngungversuches sind die Erträge wesentlich gesteigert worden, was aber darauf zurückzuführen sein dürfte, daß die Bodensäure dieser Flächen zum Teil neutralisiert worden ist.

Bor ist das interessanteste von den Elementen, deren Wirkung in Rothamsted studiert worden ist. Fräulein *K. Warington* wies nach, daß Pferdebohnen nur zur vollen Entwicklung kommen, wenn sich eine Spur von Bor in der Nährlösung befindet. Ersatz des Bors durch ein anderes Element führte nicht zum Erfolge. Die besten Ergebnisse werden erzielt, mit einer Konzentration von 1 Teil H_3BO_3 auf 1 Million Teile Lösungsmittel. Höhere Konzentrationen als 1 : 5000 erwiesen sich als schädlich. Bor scheint in irgendeiner Weise mit der Kalkaufnahme der Pflanze verknüpft zu sein. Die auffälligsten Wirkungen übt dieses Element aber auf die Knöllchenentwicklung der Leguminosen aus: Nachdem die Bakterien in die Wurzeln eingedrungen sind und mit der Vermehrung begonnen haben, bildet sich normalerweise vom Hauptleitbündelsystem der Wurzel her ein Verzweigungssystem aus, das die Gewebe, in denen sich die Bakterien befinden, knäuelförmig umgibt. Diesen Verzweigungen fällt es zu, für die Ernährung der Bakterien mittels Kohlehydraten und anderen Stoffwechselprodukten der Pflanze zu sorgen und andererseits die von den Bakterien gebildeten Stickstoffverbindungen abzuleiten. Fräulein *W. E. Brenchley* und Herr *H. G. Thornton* haben nun gezeigt, daß sich diese Verzweigung der Gefäßbündel nur selten ausbildet, wenn es an Bor fehlt. Die Bakterien erhalten dann keine Energiezufuhr von der Pflanze und entwickeln sich infolgedessen zu schädlichen Parasiten der Wirtszelle, von deren Protoplasma sie zu leben genötigt sind. Von den Nichtleguminosen braucht die Melone Bor, die Getreidepflanzen scheinen es nicht zu benötigen. *W. E. Brenchley* und *K. Warington*[1] konnten jedenfalls Gerstenpflanzen zu normaler Entwicklung bringen ohne dieses Element. In Kalifornien[2] wurde jedoch gefunden, daß kleine Mengen Bor nötig sind. Citronen scheinen Bor zu brauchen. Keinerlei derartige Mangelerscheinungen waren zu beobachten, wenn mit Stallmist gedüngt worden war.

c) Stallmist hat eine große Pufferwirkung im Boden: Er schwächt die schädlichen Wirkungen der Bodensäure erheblich ab, auch vermindert er die Säure dadurch, daß die Menge austauschfähigen Calciums im Boden vermehrt oder dieses vor Verlusten schützt. Dadurch wird die Bildung von Bodensäure verzögert. Das zeigen besonders gut die betreffenden Teilstücke der Flächen mit ununterbrochenem Anbau von

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[1] Ann. of Bot. **4**, 1—21 (1927).

[2] *A. L. Somer* and *C. B. Lipman*, Plant Physiologist **1**, 231 (1926) — Phytopathology **20**, 855 (1930).

Weizen und Gerste in Woburn. Die Flächen sind einer gleichartigen Behandlung seit 1876 unterworfen.

Übersicht 22. *Woburn, ununterbrochener Anbau von Weizen und Gerste. Einfluß der Düngung auf den Grad der Bodenversäuerung und die Menge des austauschfähigen Calciums im Boden. Milligrammprozent Äquivalente in den obersten 22,5 cm.*

	p_H		Austauschfähiges CaO		Austauschfähiges H [Grad der Ungesattigtheit]	
	Gerste	Weizen	Gerste	Weizen	Gerste	Weizen
Stallmist	5,8	6,3	6,44	6,06	3,6	3,8
Ungedüngt	5,4	5,1	3,90	4,42	5,0	5,2
Volldüngung mit Natronsalpeter.	6,1	6,0	5,46	5,24	4,1	4,4
Volldüngung mit schwefels. Amm.	4,6	4,1	1,56	1,82	5,5	8,0

Gibt es vielleicht noch andere Wirkungen des Stallmistes? Von Zeit zu Zeit wird die Ansicht geäußert, bestimmte organische Verbindungen hätten die Fähigkeit einer unmittelbaren Stimulierung des Pflanzenwachstums, die Pflanzenphysiologen sprechen von „Auximonen", die eine ähnliche Rolle spielen sollen wie die Hormone der Tierphysiologen. Wie man sich diese Erscheinung auch zu erklären haben mag, Tatsache ist, daß Zufuhr von organischen Substanzen zum Boden eine beträchtliche Steigerung des Wurzelwachstums zur Folge hat. Vorläufig ist es noch nicht gelungen, den wirksamen Bestandteil zu isolieren, aber es ist durchaus möglich, daß im Stallmist oder seinen Zersetzungsprodukten Stoffe enthalten sind, die eine derartige Wirkung ausüben.

Die Beeinflussung des Ernteertrages durch Witterungsschwankungen.

Wir wenden uns nun einem Fragenkomplex zu, der von großem wissenschaftlichen Interesse ist, dessen praktische Bedeutung aber noch weit größer sein dürfte. Eine der größten Schwierigkeiten für den Landwirt bildet die Unsicherheit seiner Ernten, die Ertragsschwankungen von Jahr zu Jahr. Wie sorgfaltig er auch die Bestellarbeiten verrichten mag, ungünstige Witterungsverhältnisse können ihm seine ganze Mühe unlohnend machen, indem die Erträge nicht in der zu erwartenden Höhe ausfallen. Deshalb beschäftigen wir uns mit der Untersuchung der Frage: Welchen Einfluß üben Witterung und Klima auf die Ertragsbildung aus? Es hat sich herausgestellt, daß Einfluß von Witterung und Düngung eng miteinander verknüpft sind. Die Untersuchung läßt sich folgendermaßen gliedern:

1. Die Beziehung zwischen Düngeweise und jährlichen Ertragsschwankungen.

2. Die Beziehung zwischen Ertragshöhe und Witterungseinflüssen (wie Niederschlagsmenge, Temperatur, Anzahl Sonnenscheinstunden).

3. Physiologische Untersuchungen die dem eingehenderen Studium der obengenannten Beziehungen dienen. Diese befinden sich noch in ihren Anfangsstadien.

Die Ertragsdaten der klassischen Einfelderversuche sind in ganz hervorragender Weise für statistische Untersuchungen dieser Art geeignet. Die Untersuchungen wurden durchgeführt von *R. A. Fisher* und seinen Mitarbeitern. *Fisher* entwickelte zunächst die speziellen Methoden zur Behandlung dieser „kurzfristigen" Versuchsreihen, denn, wenn 60—70 Jahre auch eine lange Zeit für den praktischen Landwirt bedeuten, für den Statistiker ist es eben nur eine kleine Spanne; er würde am liebsten mit noch viel längeren Zahlenreihen operieren können. Die von ihm entwickelten Methoden sind unter der Bezeichnung „Analyse variabler Größen" allgemein bekannt geworden. Er und sein Stab von Mitarbeitern gingen also daran, das Feldversuchsmaterial unter den in Frage stehenden Gesichtspunkten zu analysieren.

Der Einfluß der Witterung auf den Ertrag.

Die Verringerung der Ertragsschwankungen durch Stallmistdüngung ist sicherlich zum Teil auf den Nährstoffgehalt des Stallmistes zurückzuführen, kann diese Wirkung doch bis zu einem gewissen Grade auch mit Hilfe künstlicher Düngemittel erzielt werden. Die Ausmaße der mittleren Jahresschwankungen sind für die wichtigsten Parzellen vergleichsweise zusammengestellt in Übers. 23.

Übersicht 23. *Durchschnittliche jährliche Ertragsschwankungen.*

a. Broadbalkfield. Ununterbrochener Weizenbau seit 1843.

(Nach *R. A. Fisher*[1].)

Parz.-Nr.	Düngung	Relative[2] Jahresschwankung	Relativer Anteil der Witterungsfaktoren an den jährlichen Schwankungen	Prozentualer Anteil der Jahresschwankungen durch Witterungsfaktoren %	Durchschnittlicher[3] jährlicher Ertragsrückgang %
2	Stallmist	100	47	47	0,09
8	Volldüngung [3 N] .	150	47	31	0,26
7	„ [2 N] .	185	51	27	0,46
6	„ [1 N] .	191	66	35	0,62
5	Mineralstoffe [— N)	179	52	29	0,63
13	Volldüngung	135	45	33	0,41
11	Ohne Kali	412	87	21	0,99
3	Ungedüngt	204	90	31	0.79

[1] *R. A. Fisher*, J. agricult. Sci. **11**, 107 (1921) — Phil. Trans. B **213**, 89 (1924).

[2] Relative Jahresschwankung $= \frac{\Sigma d^2}{Y^2} \cdot K$, worin d = Abweichung des betreffenden Einzeljahrertrages von dem entsprechenden Punkt einer durch Berechnung ausgeglichenen Kurve bedeutet.

Y = Durchschnittsertrag.

K = Konstante, die so gewählt worden ist, daß die relative Jahresschwankung der Stallmistparzelle = 100 wird.

[3] Der durchschnittliche jährliche Ertragsrückgang ist ausgedrückt in Prozenten des Durchschnittsertrages.

b) *Kartoffeln: Erträge der Sorte Kerrs Pink* (dz/ha).

Düngung	1922	1923	1924	1925	1926	Größte Differenz
Volldüngung + Stallmist	240	314	222	—	—	92
Volldüngung	209	307	183	233	286	124
Ohne Kali	62	244	156	126	237	182

c) *Gerste: Erträge der Sorte Plumage Archer* (hl/ha).

Düngung	1922	1923	1924	1925	1926	Größte Differenz
Volldüngung	32,2	31,6	26,8	28,0	41,2	14,4
Ohne Stickstoff	27,9	17,9	19,9	22,5	43,1	25,2

Die Stallmistparzelle schneidet am besten ab, gleich danach kommt die Volldüngungsparzelle mit 3facher Stickstoffgabe. (Die Fläche erhält jedes Jahr eine Gabe von 144 kg/ha N in Form von 670 kg/ha schwefelsaurem Ammoniak.) Die Fläche mit normaler Stickstoffgabe zeigt schon größere Ertragsschwankungen, am weitesten sind die Schwankungen aber auf der Fläche ohne Kalidüngung. Die Reihenfolge nach dem Grade der Ertragsschwankungen ist dieselbe wie die nach Ertragsnachlaß, und die beiden Größen sind zweifellos eng miteinander verknüpft.

Allgemein haben wir die Erfahrung gemacht: Je vollständiger das Gemisch von Nährstoffen ist, das der Pflanze verabreicht wird, um so geringer sind die Ertragsschwankungen von einem Jahr zum anderen. Wenn wir daher imstande wären, die optimale Mischung zu finden, so sollte man erwarten, daß die Schwankungen auf ein Mindestmaß herabgedrückt werden würden. Die absolute Ertragshöhe würde dann einzig und allein vom Bodentyp bestimmt werden. Alle bisher geprüften Feldfrüchte zeigen in dieser Beziehung gleiches Verhalten. Die Verhältnisse bei Weizen und Kohlrüben sind schon vorgeführt worden, andere Beispiele geben die Futterrüben- und Kartoffelerträge ab. Je besser das Düngergemisch ist, desto gleichmäßiger sind die Erträge von einem Jahr zum andern. Auslassen eines einzigen Nährstoffes macht den Ertrag unsicher. Diese Ergebnisse sind bildlich dargestellt in Abb. 6, in der auch die Gesamtschwankungen der Erträge zum Ausdruck kommen. In diesem Falle ist der mittlere jährliche Ertragsrückgang Verschlechterungen der Bodenverhältnisse zuzuschreiben. Mit Gerste sind ähnliche Ergeb-

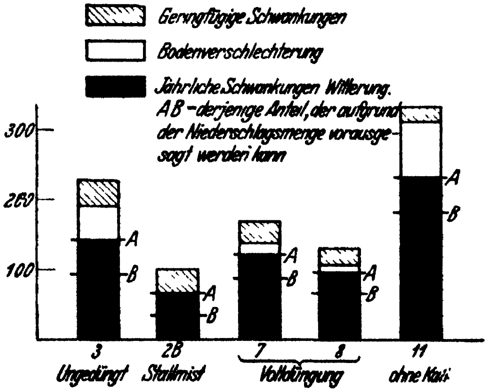

Abb. 6.

nisse gezeitigt worden. Die Schwankung ist bei den Volldüngungsparzellen nicht wesentlich verschieden von der bei den entsprechenden Weizenflächen. Bei Auslassen eines Bestandteiles aus dem Volldüngungsgemisch vergrößern sich die Schwankungen jedoch in höherem Maße als beim Weizen[1].

Man kann dasselbe auch noch in anderer Weise ausdrücken, indem man sagt: Gewöhnlich hat ein Düngemittel seine größte Wirkung in einem schlechten Jahr. So brachten die mit Kali versorgten Parzellen des Weizenversuchs auf Broadbalkfield in Jahren mit ungünstigen Witterungsverhältnissen immer beträchtlich bessere Erträge als die nicht mit Kali versehenen. Die Ertragsverhältnisse der ungedüngten Fläche geben dabei den Maßstab zur Beurteilung des Jahrescharakters ab. Nachfolgende Übersicht bringt einen Vergleich zwischen günstigen und ungünstigen Jahren zur Anschauung.

Übersicht 24. *Broadbalkfield-Rothamsted. Einfluß der Witterung auf die Ertragsbeeinflussung bei Weizen durch Kalidüngung* (Weizenerträge in kg/ha).

Parz. Nr.	Düngung	In 9 schlechten[2] Jahren		In 9 guten[3] Jahren	
		Korn	Stroh	Korn	Stroh
4	Ungedüngt	616	974	986	1210
11	Nicht genügend Kali. . . .	1187	2083	1691	2464
13	Genügend Kali	1904	3382	2218	3539
Ertragszuwachs durch Kali (Proz.) .		60,3	62,3	31,1	43,6

In den schlechten Jahren (Erntejahre: September—August) betrug die durchschnittliche jährliche Niederschlagsmenge 826,8 mm, in den guten fielen im Durchschnitt nur 688,3 mm Regen. Die Ungunst der Witterung mag gerade auf hohen Niederschlägen und den damit verbundenen niedrigen Temperaturen beruhen. Ähnliche Ergebnisse erhält man auch, wenn andere ungünstige Bedingungen an Stelle der eben genannten eintreten.

Die Witterungsfaktoren.

Unter diesen ist die Niederschlagsmenge von größter Bedeutung. Je nach Düngeweise sind die klimatischen Einflüsse ihrer Wirkung nach verschieden. Es scheint danach so, als gäbe es eine Düngeweise, die allen anderen an Wirksamkeit überlegen ist. Vorläufig sind Gerste und Weizen bezüglich ihres Verhaltens gegenüber dem Wetter nur auf unserem schweren Boden in Rothamsted eingehender untersucht worden, wir beginnen aber jetzt damit, sie auch auf dem leichten Sandboden von Woburn daraufhin zu prüfen. Die Weizenflächen mit Volldüngung (N P K) leiden stark unter den Regenfällen während des Winters. Auf den Stallmistflächen ist der nachteilige Einfluß schwächer. Für Weizen und Gerste liegen die Verhältnisse im großen und ganzen etwa folgendermaßen:

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[1] *M. A. Mackenzie,* J. Agricult. Sci. **14**, 434 (1924).

[2] Die schlechten Jahre waren: 1867, 1871—1872, 1875—1877, 1879, 1886 und 1888.

[3] Die guten Jahre waren: 1868—1870, 1881, 1883, 1885, 1887, 1889 und 1891.

Einfluß der Niederschlagsmege auf die Ernte[1].

	Aussaat	Okt.—Dez.	Dez.—März	April—Mai	Juni—August
Weizen. .	Oktober	schwach schädlich	schädlich	schwach schädlich	schädlich
Gerste . .	März	förderlich	schwach schädlich	schädlich	ziemlich förderlich

Der Einfluß von Regenfall wahrend der Wintermonate kommt zum Ausdruck in Übersicht 25.

Übersicht 25. *Broadbalkfield-Rothamsted. Ununterbrochener Anbau von Weizen. Durch* 25 *mm übernormale Regenmenge während der Monate November bis Januar hervorgerufener Höchstverlust an Ertrag* (hl/ha).

Parz. Nr.	Düngung	Durchschnittl. Jahresertrag (74 Jahre)	Mittlerer Höchstverlust	
			hl/ha	%
3 u. 4	Ungedungt.	10,5	0,36	3,4
5	Mineralstoffe	12,1	0,27	2,2
6	Mineralstoffe + 48 kg N	19,5	0,99	5,1
7	„ + 96 kg N	27,4	1,35	4,9
8	„ +144 kg N	31,1	1,35	4,3
10	„ + 96 kg N	16,8	0,81	4,8
2	Stallmist.	30,1	0,90	3,0
11	Ohne Kali	19,2	0,81	4,2
13	Volldüngung	26.2	1.62	6.2

Diese Ergebnisse sind illustriert in Abb. 7.

Der Verlust an Erntemasse schwankt, je nachdem zu welchem Zeitpunkt der Regen fällt. Die Daten in der Übersicht geben aber Anhaltspunkte dafür ab, zu welchem Zeitpunkt die größten Benachteiligungen für die Ernte aus zuviel Feuchtigkeit zu erwachsen pflegen. Je größer die Stickstoffdüngung, desto geringer ist im allgemeinen die relative Einbuße an Ertrag, wenn auch der absolute Ausfall an Erntemasse größer ist. Man kann sagen, daß 25 mm Regen über den Durchschnitt innerhalb der Zeit von November bis zum Januar den Ertrag im Mittel um etwa 1,3 hl verringern. Eine Gabe von 48 kg N steigert den Ertrag um 6 hl, so daß man rechnen kann, daß 10 kg N nötig sind, um die Ertragsdrückung durch 25 mm Winterregen aufzuwiegen. Das ist etwa das

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[1] *R. A. Fisher*, Phil. Trans. B **213**, 89 (1924). — *J. Wishart* and *W. A. Mackenzie*, J. Agricult. Sci. **20**, 417 (1930).

Doppelte der Menge N, die gewöhnlich durch Auswaschung verloren geht. Ebenso geht aus den Versuchen von Woburn hervor, daß ein Auswaschungsverlust von einem Teil Stickstoff einen ebenso hohen Ernteverlust verursacht wie Fehlen von 2 Teilen in der Düngung.

Bei Gerste ist die Regenmenge, die während der 6 Monate fällt, bevor das Korn in den Boden kommt, ebenso bedeutsam wie die, welche während der Vegetationszeit fällt. Die Wirkung der in den einzelnen Monaten fallenden Menge ändert sich jedoch je nach Düngeweise. Die Pflanzen der Kalimangelparzellen 2—0 und 2—A (vgl. Abb. 8) scheinen ganz besonders stark unter nassen Wintern zu leiden, die auf der Phosphorsäuremangelfläche 3—A leiden andererseits besonders stark unter nassen Frühjahren.

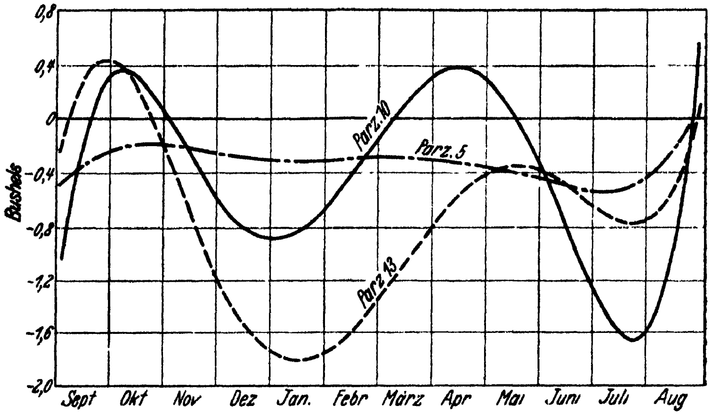

Abb. 7.

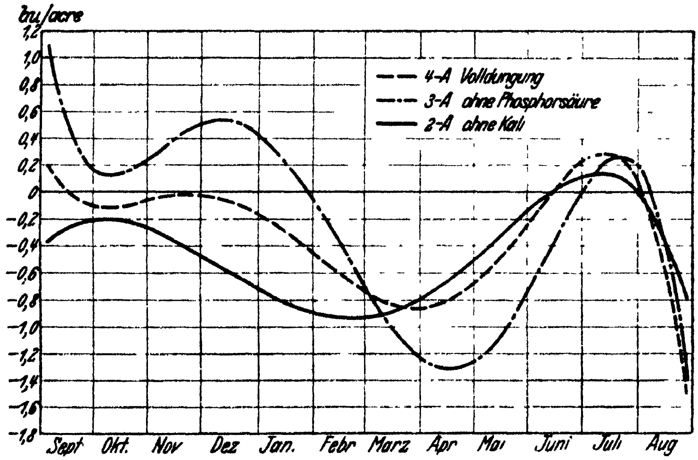

Abb. 8.

Der Einfluß der Temperatur ist weniger bedeutsam als der der Regenmenge. Er spielt aber eine große Rolle während der Jugendentwicklung der Pflanzen. Nach unseren Erfahrungen verkürzt Zunahme der Bodentemperatur um 1 ° F (0,55 ° C) in Rothamsted die Zeit zwischen Aussaat und Auflaufen bei Sommergetreide im Durchschnitt um einen Tag, bei Wintergetreide um $1^1/_2$—2 Tage. Kohl- und Wasserrüben werden dagegen von der Bodentemperatur kaum beeinflußt, da zur Zeit ihrer Aussaat die Temperaturen schon zu weit angestiegen zu sein pflegen. Die jährlichen Schwankungen in der Anzahl Sonnenscheinstunden beeinflussen den Weizenertrag in Rothamsted viel weniger als die Schwankungen in der Niederschlagsmenge. Die Daten für Kartoffeln sind unzulänglich für exakte statistische Behandlung. Beim Überblicken will es jedoch so scheinen, als ob der Einfluß von Schwankungen in der Sonnenscheindauer nicht groß ist, sofern reichliche Zufuhr aller Nährstoffe gewährleistet ist. Fehlt dagegen das Kali in der Düngung, so gewinnt dieser Umwelteinfluß wesentlich an Bedeutung.

An derartige Fragen knüpfen sich eine Reihe wichtiger praktischer Folgerungen. Ist der Witterungscharakter einer Gegend bekannt, so können allen denjenigen, die mit der Durchführung von Versuchen betraut sind, Fingerzeige gegeben werden, mit welchen Düngemitteln und Gemischen von Düngemitteln sie arbeiten sollten, um für die Landwirte des betreffenden Kreises die geeignetste Düngeweise zu ermitteln. Am verlockendsten aber wäre es, wenn man Richtlinien für eine Versicherung gegen Ernteverluste durch Witterungseinflüsse aufstellen könnte. Ebensowenig wie jemand voraussehen kann, ob er am Ende des Jahres noch lebt, kann ein Landwirt bei der Aussaat wissen, mit welchen Erträgen er rechnen kann. Die Versicherungsstatistiker haben aber Tafeln ausgearbeitet über die mutmaßliche Lebensdauer von Menschen in bestimmtem Alter. Diese Berechnungen führten zur Gründung von Versicherungsgesellschaften und gaben, sofern die Beteiligung einer genügend großen Anzahl von Interessenten für eine Lebensversicherung vorhanden war, die Grundlage ab für eine heute allgemein geschätzte Einrichtung. Wir sind jetzt damit beschäftigt, entsprechende Tafeln für die mutmaßliche Höhe von Ernteerträgen aufzustellen, die, wie wir hoffen, zur Gründung von Versicherungsgesellschaften gegen Ernteverluste durch Witterung führen werden. Erste Bedingung für Beitritt zu dieser Versicherung soll sein, daß die Landwirte vorschriftsmäßige Düngung innehalten. Die großen Düngemittelsyndikate werden voraussichtlich die Herstellung geeigneter Düngergemische dann übernehmen. Die Prämien können nicht sehr hoch ausfallen, die für den Landwirt dadurch geschaffene Erleichterung dürfte dagegen sehr groß sein, wenn er die Beruhigung hat, unabhängig von Witterungsunbilden wirtschaften zu können.

Witterungsbedingungen und Wirksamkeit von Düngemitteln.

Zu dem Zwecke, bessere Einsicht in die Wirkung von Witterungseinflüssen auf den Grad der Wirksamkeit von Düngemitteln zu erhalten, wurde in Rothamsted auf Long Hoosfield ein neuer Fruchtfolgeversuch auf 30 Jahre angelegt. Die Fruchtfolge ist sechsfeldrig: Gerste — Rotklee — Weizen — Kartoffeln — Futtergemenge (Roggen, Pferdebohnen und Wicken), danach Senf, später Roggen, welche letzteren beiden untergepflügt werden, — Zuckerrüben. Die für jede einzelne Frucht zur Verfügung stehende Fläche ist in 5 gleiche Teile geteilt, die steigende N-Gaben erhalten. Eine Fläche bleibt ungedüngt, so daß 4 um je 1 Einheit steigende Dosierungen zur Anwendung kommen. Über die gegenseitige Lage dieser Flächen zueinander bestimmt der Zufall. Ebenso sind je 4 Flächen mit steigender K- und mit steigender P-Düngung im Versuch vorgesehen. Jede Gruppe von Teilstücken erhält von vornherein eine Grunddüngung. Jedes Teilstück erhält im jeweils nachfolgenden Jahre 1 Einheit des gleichen Düngemittels weniger als es im vorhergehenden erhalten hatte, und zwar solange, bis das betreffende Düngemittel ganz ausgelassen wird. Danach erhält es 1 Jahr lang 4 Einheiten des 2. Düngemittels, dann 1 Jahr nur 3 Einheiten und so fort, bis auch dieses Düngemittel weggelassen wird. Ebenso wird mit dem 3. Düngemittel weitere 5 Jahre hindurch verfahren. Auf diese Weise soll es vermieden werden, daß infolge Anhäufung von Nährstoffen usw. im Boden der Versuch unliebsam beeinflußt wird. Im 1. Jahre erhalten die 5 Teilstücke der Stickstoffgruppe z. B.:

4, 3, 2, 1, 0 Einheiten N + je 2 Einheiten K und P

im 2. Jahr wechselt es dann für die ersten 4 Teilstücke:

3, 2, 1, 0 Einheiten N + je 2 Einheiten K und P.

Für das 5. Jahr betragen die Gaben 4 Einheiten K (bzw. P) + 2 Einheiten der beiden anderen (P + N bzw. K + N).

Nach Verlauf von 15 Jahren ist dieser Zirkel dann einmal geschlossen und jedes Teilstück erhält wieder die gleiche Düngung wie im 1. Jahr. Dann ist die 2. Rotation im Anbau der Früchte gerade zur Hälfte beendet. Nach 30 Jahren, wenn der Düngezirkel 2 mal gekreist hat, sind 5 volle Turnus der Fruchtfolge vollendet, und es kann von neuem beginnen (vgl. den Versuchsplan).

In Wiederholungen sind die Teilstücke nicht angelegt worden. Der Versuchsfehler kann aber durch Vergleich der tatsächlich gefundenen Kurve — darstellend das Verhältnis von Düngermenge zu Ertrag — und der theoretisch zu erwartenden — durch eine Ausgleichsrechnung jedesmal ermittelten — Kurve annäherungsweise festgestellt werden. Die Einzeljahresergebnisse werden schon wertvolle Anhaltspunkte geben, die Besprechung kann jedoch in ihrem vollen Umfang erst nach Abschluß des Versuches im Jahre 1961 vorgenommen werden.

Versuchsplan. Neue sechsfeldrige Fruchtfolge auf Long Hoosfield (1930—1961).

B W (Parz. 1—15) Weizen.

1 3 P	2 0 P	3 0 N	4 4 K	5 2 K
6 4 N	7 2 P	8 3 N	9 0 K	10 1 K
11 1 P	12 2 N	13 1 N	14 3 K	15 4 P

B S (Parz. 16—30) Zuckerrüben.

16 3 N	17 4 P	18 2 P	19 3 P	20 3 K
21 0 N	22 2 N	23 1 P	24 0 K	25 4 N
26 1 N	27 0 P	28 4 K	29 2 K	30 1 K

B B (Parz. 31—45) Gerste.

31 2 K	32 0 K	33 0 P	34 2 P	35 3 N
36 3 K	37 1 K	38 4 N	39 4 K	40 0 N
41 4 P	42 3 P	43 1 P	44 2 N	45 1 N

B C (Parz. 46—60) Klee.

46 3 P	47 0 P	48 1 K	49 4 N	50 2 N
51 1 P	52 4 K	53 2 K	54 3 N	55 1 N
56 2 P	57 0 K	58 3 K	59 0 N	60 4 P

B P (Parz. 61—75) Kartoffeln.

61 4 P	62 0 K	63 1 P	64 0 P	65 1 N
66 3 K	67 1 K	68 2 P	69 0 N	70 4 K
71 2 K	72 3 P	73 4 N	74 2 N	75 3 N

B F (Parz. 76—90) Futtergemenge.

76 4 K	77 0 P	78 3 K	79 0 K	80 0 N
81 2 P	82 3 P	83 4 N	84 2 N	85 3 N
86 1 P	87 2 K	88 1 K	89 4 P	90 1 N

Die Erzeugung von Viehfutter.

Augenblicklich sind in England die wirtschaftlichen Bedingungen ungünstig für den Ackerbau. Gras wächst dagegen leicht und ist billig zu gewinnen. Infolgedessen gestaltet sich die Viehhaltung während der Sommermonate viel einfacher. Nur die Winterfütterung macht Schwierigkeiten. Deshalb machen es viele Landwirte so, daß sie im Herbst ihr Vieh absetzen, um im Frühjahr erst wieder welches einzustellen. Nun ist gegenwärtig die Grünlandfläche immer mehr im Zunehmen begriffen, so daß auch das Angebot auf dem Viehmarkte steigt, was seinerseits die Preise im Herbst außerordentlich drückt, im Frühjahr sie dagegen über Gebühr ansteigen läßt. So begründet sich die Notwendigkeit, für Erzeugung billigen Winterfutters Sorge zu tragen.

Dafür gibt es unter englischen Verhältnissen 2 Wege: Getreide und Kraftfuttermittel sind billig, und zwar so billig, daß es sich oft gar nicht lohnt, sie selbst anzubauen. Auch Düngemittel sind nicht teuer. Es kann daher ganz vorteilhaft sein, sie in Viehfutter selbst zu verwandeln, anstatt Kraftfuttermittel einzukaufen. Diesen Fragen wollen wir jetzt näher treten.

Die Feldversuche der letzten 10 Jahre haben uns gelehrt, wie hoch der Ertragszuwachs ist, den man im allgemeinen von einem Doppelzentner schwefelsauren Ammoniaks erwarten kann, wenn der Boden im Laufe der Rotation genügend Phosphorsäure und Kali erhalten hat. Auch die chemische Zusammensetzung der gesteigerten Erntemasse ist uns bekannt. In Futtereinheiten ausgedrückt, ergeben sich folgende Werte:

Übersicht 26. *Durchschnittlicher Erntezuwachs durch 1 dz schwefelsaures Ammoniak bei Anwesenheit von genügend Phosphorsäure und Kali im Boden.*

		Erntezuwachs	Gewichtsprozentischer Zuwachs an		Absoluter Zuwachs an kg			
		dz	Protein	Stärke	Protein		Stärke	
Kartoffeln		20,0	0,6	18	12,0		360	
Futterrüben		30,0	0,4	7	13,0		225	
Kohlrüben		20,0	0,7	7	14,5		140	
Gerste:	Korn	3,0	8,5	71	28	33	232	378
	Stroh	6,5	0,7	23	5		146	
Hafer:	Korn	2,5	7,6	60	20	26	160	280
	Stroh	6,0	0,9	20	6		120	
Weizen:	Korn	2,5	9,6	72	24	30	175	241
	Stroh	5,0	0,1	13	6		66	
					Mittel 24		Mittel 280	

Untersuchungen über Bodenkultur.

Wir haben bisher nur über Fragen der Pflanzenernährung gesprochen. Die Bodenkultur ist aber ein mindestens ebenso wichtiger Faktor im Pflanzenbau wie die Düngung. Ein tüchtiger Landwirt ist ein reiner

Künstler in der Bearbeitung seines Bodens, zu einer Wissenschaft aber ist dieses Gebiet erst vor gar nicht allzu langer Zeit erhoben worden. Ja man kann sagen, in diesen Fragen stehen wir heute auf demselben Flecke wie in der Düngung vor 90 Jahren. Seit langem hat man wohl empirische Erfahrungen in Hülle und Fülle gesammelt, und auch die wissenschaftliche Seite hat sich beträchtlich fortentwickelt, aber beherrscht von Regeln, die aus dem Laboratorium stammen, wird die Bodenkultur noch immer nicht.

In Rothamsted sind es *Keen* und seine Mitarbeiter, die sich darum bemühen, die Kunst der Bodenbearbeitung zu einer Wissenschaft zu machen. Zweck der Bodenbearbeitung ist es, den Garezustand eines Bodens herbeizuführen. Die Untersuchung zerfällt somit in 2 Hauptabschnitte: 1. Welches ist die Wirkung der verschiedenen Bodenbearbeitungsgeräte, und 2. was verstehen wir unter Bodengare ? Die 1. Frage ist leichter zu beantworten. Man kann ihr von 2 Seiten zu Leibe rücken: Zuerst sind genaue Beobachtungen auf dem Felde anzustellen, und dann sind die dort gemachten Befunde im Laboratorium weiter zu bearbeiten, denn es gilt, die physikalischen Eigenschaften des Bodens kennenzulernen. Die Laboratoriumsbefunde dienen dann wieder zur Erklärung der Feldbeobachtungen.

Für Feldbeobachtungen ist das Dynamometer, wie es nach den Angaben von *Keen* und *Heines* konstruiert worden ist, eins der brauchbarsten Hilfsmittel. Dieser Apparat registriert gleichzeitig Zugkraft, mittlere Arbeitsgeschwindigkeit und alle Beobachtungen, die der begleitende Beobachter macht, in Morseschrift. Die Ablesungen von dem Celluloidstreifen werden dann noch einmal besonders gebucht und nach den gesammelten Werten Isodynenkarten angefertigt, indem Punkte mit gleicher Zugkraft untereinander durch eine Linie verbunden werden. So eine Karte zeigt dann die Verteilung von Stellen mit leichterem und schwererem Boden auf dem Felde ganz in derselben Weise an, wie gewöhnliche Landkarten Tiefland und Gebirge.

Isodynenkarten eines Feldes, die nach Messungen in verschiedenen Jahren angefertigt sind, zeigen kaum Unterschiede, obwohl die numerischen Werte der Widerstandsmessungen je nach Feuchtigkeitsgehalt und Bodenstruktur veränderlich sind. Die Feldbeobachtungen werden danach mit diesen Aufzeichnungen verglichen. Dabei hat es sich gezeigt, daß die benötigte Zugkraft sich fast gleichlaufend mit dem Gehalt des Bodens an Ton verändert. Auch die Anzahl von Weizenpflanzen, die den Winter überdauern, steht in enger Beziehung zur erforderlichen Zugkraft, und zwar ist sie am höchsten an den Stellen mit leichterem Boden und am niedrigsten dort, wo der Boden besonders schwer ist. Zum endgültigen Ernteertrag bestehen allerdings keine derartig engen Beziehungen. Die mehr vereinzelt wachsenden Pflanzen auf dem schweren und stärker absorbierenden Böden haben höhere Erntegewichte als die enger stehenden auf den leichteren Stellen, wodurch die Unterschiede wieder nahezu ausgeglichen werden. Dagegen bestehen wieder Zusammenhänge zwischen Zugkraft und Menge des abfließenden Drainwassers, und zwar ist Menge des letzteren am größten an Stellen mit hohem Widerstand gegenüber dem Pflug, oder, was dasselbe ist, mit hohem Tongehalt im Boden. Das Wasser muß auf diesen Stellen durch die Drainrohre abfließen, weil es nicht so leicht im Boden versickern kann. Ist der Boden nicht drainiert, so stagniert es an diesen Stellen[1].

Mit Hilfe des Dynamometers können in gleicher Weise auch von der Beschaffenheit des Untergrundes Karten angefertigt werden. Die so gewonnenen Werte werden dann mit Laboratoriumsuntersuchungen in Einklang gebracht und mit mechanisch-physikalischen Bezeichnungen belegt, ähnlich, wie ein Ingenieur seine Fachfragen begrifflich in eine besondere Sprache kleidet, sie in dieser Weise durchdenkt und löst.

In diesem Zusammenhang wurden vornehmlich gemessen 1. die Kohäsion — nach der etwas veränderten Atterbergschen Methode —, 2. die Reibung, die an der metallenen Oberfläche (des Pflugschars) beim Durchfurchen des Erdbodens entsteht, und 3. die Plastizität des Bodens. Namentlich die letztgenannten Untersuchungen haben sich als fruchtbar erwiesen. Was man in diesem Falle mißt, ist die Viscosität oder vielmehr die Pseudoviscosität von breiartigen Bodenaufschwemmungen. Die Energiemenge, die gerade ausreicht, den betreffenden Bodenbrei zum Fließen zu bringen, wird nach *Keen*[2] als seine „statische Steifheit“ bezeichnet. Zwischen diesem Wert und der Widerstandsmessung mittels Dynamometer bestehen enge Beziehungen.

Bisher ist hauptsächlich versucht worden, die inneren Zusammenhänge zwischen den verschiedenen physikalischen Bodeneigenschaften aufzudecken sowie deren Beziehungen zum Sand- und Tongehalt des Bodens festzustellen. Das ist aber nötig, um sich über die Bedeutung der gefundenen Werte klar zu werden.

Besonders interessante Ergebnisse haben Untersuchungen gehabt, die sich mit dem Einfluß einer Düngung von kohlensaurem Kalk auf die Bodenstruktur befaßten. Heftige Kalkung (500 dz/ha), wie sie noch bis vor gar nicht allzu langer Zeit in der Praxis auf den Böden Südostenglands vorgenommen wurde, wo die Kreide ansteht, ja sogar stellenweise zutage tritt, vermindern den Widerstand, den der Boden dem Pflugschar entgegensetzt, um ein Beträchtliches. Kleine Gaben (25 dz/ha), wie sie jetzt mehr üblich geworden sind, üben keine derartige Wirkung aus.

Daneben wurden die Wirkungen der Bodenbearbeitungsgeräte untersucht. Durch Aussieben von Boden wurde die Größe der Bodenklümpchen und deren Verteilung im Boden ermittelt. Die Ergebnisse derartiger Untersuchungen können entweder ausgedrückt werden als relative Häufigkeit der Klümpchen von verschiedener Größe oder als Summe aller Oberflächen der Einzelklümpchen. Bei einer Untersuchung über den Einfluß gewöhnlichen Pflügens auf einen gewachsenen Boden einerseits und von Behandlung desselben mit der Bodenfräse andererseits ergaben sich nachstehende Werte:

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[1] *W. B. Heines* and *B. A. Keen*, J. Agricult. Sci. **15**, 387 (1925).

[2] J. Agricult. Sci. **15**, 395 (1925).

Relative Häufigkeit von Klümpchen verschiedener Größe	Unbehandelt	Gepflügt	Gefräst
Groß	60	45	30
Mittel	33	40	55
Klein	7	13	13
Summe der Oberflächen	320	475	530

Die Summe aller Oberflachen wird dagegen nur wenig verändert, wenn das Bodengefüge von vornherein lockerer ist.

Auch der 2. Fragenkomplex, der vom Wesen und von der Bedeutung der Bodengare handelt, ist in Angriff genommen worden. Allgemein gesprochen, bedeutet Bodengare einen Zustand, in dem ein Boden sich befindet, wenn er eine feine krumige Beschaffenheit aufweist, weder zu schmierig noch zu pulverig ist. Dieser Zustand steht vielleicht im Zusammenhang mit der elektrischen Ladung der allerfeinsten Tonteilchen.

Die neue Feldversuchs-Methode.

Die alten Rothamsteder Feldversuche sind so angelegt, daß die einzelnen Teilstücke in ganz bestimmter Ordnung eins neben dem anderen zu liegen kommen, z. B. auf Broadbalkfield, oder sie liegen in einer Reihe nebeneinander und werden noch quer zu dieser Richtung verschiedenartig behandelt, wie es für Hoos- und Barnfield zutrifft. Diese Art der Anlage hatte den großen Vorzug der Einfachheit und unmittelbaren Übersichtlichkeit; sie war daher auch ganz besonders zu Schauversuchen für die Landwirte geeignet. Bei sorgfaltiger Durchführung erhält man mit derartigen Versuchen auch gute Ergebnisse, zumal wenn sie lange Jahre hindurch wiederholt werden wie in Rothamsted. Im übrigen ist die Methode aber nicht sehr exakt, und der Versuchsfehler kann alljährlich bis zu 10 % betragen. Der größte Nachteil der Methode besteht freilich darin, daß sie keine Handhabe zur Ermittlung dieses Versuchsfehlers liefert: Der Grad der Genauigkeit der erzielten Versuchsergebnisse kann daher nicht angegeben werden.

Die statistische Abteilung wurde 1919 eingerichtet und der Leitung *R. A. Fishers* unterstellt. Aufgabe dieser Abteilung war es, die ungeheure

Fülle von Feldversuchsergebnissen, meteorologischen Aufzeichnungen und Laboratorienbefunden, die sich seit 1843 angehäuft hatte, zu bearbeiten und kritisch zu sichten. Neue Versuche wurden nötig, um zweifelhafte Fälle zu entscheiden. Diese neueren Versuche wurden so angelegt, daß statistische Methoden leicht auf sie angewandt werden können. Dazu sind folgende Erfordernisse nötig: 1. Die einzelnen Parzellen müssen in genügend großer Anzahl von Wiederholungen angelegt sein und 2. dürfen sie nicht eine willkürliche, von vornherein festgelegte Reihenfolge innehalten, sondern müssen beliebig, nach dem Gesetze des Zufalls, über die gesamte Versuchsfläche verteilt sein.

R. A. Fisher begann 1919 damit, zum Studium statistischer Fragen seine analytische Methode zur Untersuchung variabler Größen auszuarbeiten, die gegenüber der gewöhnlichen Korrelationsmethode mancherlei Vorteile aufweist. So schaltet sie die Berechnung einer großen Anzahl von Zwischenwerten, die für die eigentliche Untersuchung bedeutungslos sind, aus. Auch werden eine Reihe sonst notwendiger Berichtigungen der errechneten Daten unnötig gemacht, was besonders bei kleinen Versuchsserien sonst schwer zu umgehen war. Er wandte seine analytische Methode auf die Ertragsdaten von Broadbalkfield an und konnte damit den Einfluß bestimmter Faktorenkomplexe zerlegen, die bei der Ertragsbildung mitspielen. Diese Untersuchungen ließen so recht erkennen, wie groß das Bedürfnis für exaktere Methoden zur Untersuchung kleiner Versuchsserien ist. Und gerade durch die enge Begrenztheit ihres Umfanges sind ja die landwirtschaftlichen Versuche im allgemeinen gekennzeichnet. Das erste Beispiel für eine Untersuchung modernen Stils bildete der von *T. Eden* 1922 durchgeführte Düngungsversuch mit verschiedenen Kartoffelsorten[1]. Etwas später brachte „Student“ eigene Untersuchungen zusammen mit solchen von *Fisher* über die Anwendbarkeit von Formeln heraus, die zur Verrechnung von Getreidesortenversuchen dienen sollten. So wurden allmählich streng wissenschaftliche Methoden ausgearbeitet, die sich auf das landwirtschaftliche Versuchswesen anwenden lassen.

Als nächster Schritt folgte der Ausbau einer ebenso exakten Feldversuchstechnik. In diesem Zusammenhange sind die Namen *T. Edens, E. J. Maskells* und allen voran *Fishers* zu nennen. Die Hauptschwierigkeit bestand in der Beseitigung der unerwünschten Beeinträchtigungen von Versuchsergebnissen durch Bodenunterschiede, ein Hemmnis, das bei der Auswertung von Feldversuchsergebnissen so oft im Wege gestanden hatte. Zum Teil konnte diese Schwierigkeit überwunden werden durch geeignete Verteilung der einzelnen Teilstücke über die gesamte Versuchsfläche. Es blieb dabei jedoch immer noch eine ihrem Ausmaß nach unbekannte Fehlerquelle übrig. Es ließ sich nun zeigen, daß die genannten analytisch-statistischen Methoden zur gänzlichen Eliminierung der erstgenannten Einflüsse führen und es gleichzeitig möglich machen, die restlichen Fehlerquellen ihrer Größe nach zu schätzen, allerdings nur unter der Voraussetzung, daß mit einer genügenden Anzahl von Wiederholungen gearbeitet wird und diese nach dem Prinzip des Zufalls über die ganze Fläche verteilt sind.

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[1] *Fisher* u. *Mackenzie,* J. Agricult. Sci. 1923.

Es wurden dann im Laufe der Zeit verschiedenerlei Versuche zum Studium der feldversuchstechnischen Erfordernisse angestellt, die eine Bearbeitung des Versuchsmaterials nach mathematisch-statistischen Gesichtspunkten gewährleisten. Die dabei benutzten Methoden wurden dann untereinander wiederum in besonderen Versuchen auf ihre Übereinstimmung und Brauchbarkeit zu praktischen Verwendungszwecken geprüft. Besonders die beiden folgenden erwiesen sich als brauchbar. Versuchsanlagen mit ganz beliebiger Verteilung der einzelnen Teilstücke „Randomised blocks“ und das sog. „Römische Karree“ (Latin square).

Der erstere Typ ist einfach zu handhaben und läßt sich besser den jeweiligen Besonderheiten des betreffenden Versuchsfeldes und der Versuchspflanze anpassen: Die Versuchsfläche wird in verschiedene Streifen oder Blöcke eingeteilt, von denen jeder eine Parzelle der unterschiedlich behandelten Versuchsserie enthält. Die Verteilung der einzelnen Teilstücke innerhalb des Blockes ist eine ganz zufällige und wird nicht etwa schematisch vorausbestimmt. Man verfährt vielmehr in der Weise, daß man die verschiedenen Behandlungsweisen auf eine entsprechende Anzahl Karten schreibt, die Karten gründlich durchmischt und dann eine nach der anderen beliebig herauszieht. Da die einzelnen Blöcke nun nicht unmittelbar untereinander verglichen werden, gelingt es, die Bodenunterschiede auszuschalten, da andererseits die Verteilung der Parzellen innerhalb des Blockes ganz nach dem Zufall vorgenommen worden war, läßt sich mit Hilfe der Wahrscheinlichkeitsrechnung die Sicherheit der gewonnenen Werte feststellen.

Das „Römische Karee“ ist exakter, aber begrenzter in seiner Anwendbarkeit auf Düngungsversuche. Die Teilstücke sind in ebenso viel Reihen und Kolumnen angeordnet wie Unterschiede in der Behandlungsweise bestehen. Jede Unterschiedsdüngung kommt nur einmal in jeder Reihe und jeder Kolumne vor. Eine überraschend große Anzahl von Kombinationsmöglichkeiten sind möglich. Die Verteilung der Teilstücke erfolgt wiederum nach dem Prinzip des Zufalls und wird ebenso durch Mischen und Ziehen von Karten vorbereitet.

Ein weiterer Ausbau der Methode besteht in der Verschmelzung verschiedener Einzelversuche zu einem einzigen Versuch von besonders großem Ausmaß, wodurch erreicht wird, daß die Anzahl der „Freiheitsgrade“ der Versuchsergebnisse erhöht wird, exaktere Vergleiche vorgenommen werden können und die sog. Standardabweichung — die Zahl, die angibt, wie weit die Versuchsergebnisse als gesichert zu betrachten sind — genauer bestimmt werden kann. Dazu ist eine riesige Anzahl von Parzellen nötig. Einige Beispiele sind in einer Arbeit von *Wishart*[1] gegeben worden.

Welche Anzahl von Wiederholungen erforderlich ist, hängt vom Grade der Genauigkeit ab, den man anstrebt. Die Wahrscheinlichkeit für eine Ertragsdifferenz von der Größe ihrer Standardabweichung ist nur 1 : 3, selbst wenn der Versuchsansteller noch so sehr überzeugt davon ist, daß er jedem einzelnen Teilstück genau die gleiche Düngung und gleiche übrige Behandlung hat zuteil werden lassen. Eine Differenz von der Größe der doppelten Standardabweichung würde dagegen nur einmal unter 22 beliebigen Fällen auftreten. Sie ist demnach besser gesichert. Die Sicherheit von Ertragsunterschieden hängt demnach von anderen Ursachen ab als von den Unterschieden in der Behandlungsweise der Flächen. Sie beträgt:

Für Differenzen von der Größe ihrer Standardabweichung . .	3:1
„ „ „ 2mal diese Größe	22:1
„ „ „ 3mal „ „	370:1
„ „ „ 4mal „ „	15780:1

Für die meisten landwirtschaftlichen Untersuchungen genügt ein Sicherheitsgrad von 30 : 1. Die in den Übersichten dieser Arbeit angegebenen Standardabweichungen sowie die in den Rothamsteder Jahresberichten gegebenen gelten für die Ertragsdaten und müssen mit 1,414 multipliziert werden, wenn man die Standardabweichung der Differenz zwischen behandelten und unbehandelten Flächen berechnen will, worauf es ja gewöhnlich ankommt. Um eine Wahrscheinlichkeit von 30 : 1 zu haben, muß eine Differenz rund 3mal so groß wie ihre Standardabweichung sein, um für gesichert gelten zu können.

Mit unseren zur Zeit in Rothamsted benutzten Feldversuchsmethoden haben wir Standardabweichungen für das einzelne Teilstück von folgender Größenordnung:

Übersicht 27. *Übliche Standardabweichung (Fehler der Einzelbeobachtung) bei exakter Versuchsanstellung.*

		dz/ha	%
Kartoffeln		10,0[2]	7
Zuckerrüben		12,5	9
Gerste:	Korn	1,7	9
	Stroh	2,5	7
Hafer:	Korn	2,5	8
	Stroh	2,5	6

Die Standardabweichung ist der exakte Maßstab für die Genauigkeit des Versuchsergebnisses. Sie schließt ein: Die Arbeitsfehler, Ungleichartigkeiten in der Beeinflussung durch variable äußere Faktoren, wie Witterung, Vogelfraß, Insektenschäden, Pilzkrankheiten und Bodenunterschiede bei den einzelnen Teilstücken, dagegen nicht die großen Variationen von Parzelle zu Parzelle, die ja durch die Versuchanordnung ausgeschaltet werden. Daher ist eine Standardabweichung von 8 dz/ha Kartoffeln in einem „Latin square" nicht unmittelbar vergleichbar mit einer ebenso großen Abweichung in einem Versuch nach der „Randomised block"-Methode mit größerer Anzahl von Teilstücken.

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[1] Methodische Anleitung zur Auswertung von Feldversuchsergebnissen gibt *J. Wishart* im Arch. Landw., Abt. Pflanzenbau **5**, 561 (1931).

[2] In „römischen Karrees". Sonst etwa 15 dz/ha.

Nichtsdestoweniger ist es ein wertvoller Anhaltspunkt für den Grad der Genauigkeit, mit der ein Versuch durchgeführt worden ist. Die Standardabweichung ist wenig verschieden, ob die Ernte groß oder klein ist. Demzufolge haben hohe Erträge kleinere prozentische Fehler als niedrige.

Die oben gegebenen Werte sind Standardabweichungen von Einzelerträgen. Die Parzellen sind aber immer in Wiederholung angelegt, und der mittlere Fehler des Mittels, das ist die Zahl, die letzten Endes als Maßstab für die Sicherheit der gewonnenen Durchschnittserträge dient, wird errechnet, indem man den mittleren Fehler der Einzelbeobachtung dividiert durch die Quadratwurzel aus der Anzahl der zur Durchschnittsbildung zusammengefaßten Wiederholungen. Dieser „Fehler" schwankt gewöhnlich zwischen $1^1/_2$ und 3 %.

Die Möglichkeiten, den Fehler zu verkleinern, werden also fortlaufend geprüft. Ungleichmäßigkeiten entstehen durch Unregelmäßigkeiten beim Drillen oder Düngerausstreuen, besonders beim Aufbringen von Stallmist, sofern dieser im Versuch zur Anwendung kommt. Ferner werden Ungleichförmigkeiten durch verschieden starkes Auftreten von Unkraut verursacht, durch ungleich starken Insekten- oder Pilzschaden, Vogelfraß, Wildverbiß und Witterungsschäden u. a. m. Die hauptsachlichsten Ungleichmäßigkeiten werden aber hervorgerufen durch ungleichförmige Aussaat, Düngung und Verunkrautung. Wir sind standig darauf bedacht, die Versuchstechnik in dieser Beziehung zu verbessern.

Auch sind wir bestrebt, die Genauigkeit der Versuche dadurch noch weiterhin zu erhöhen, daß wir jegliche Verluste bei der Ernte oder deren Abtransport vermeiden. Dafür ist eine besondere Methode ausgearbeit worden in den Abteilungen für Pflanzenphysiologie und für Statistik. Diese Methode hat obendrein den Vorzug, den zur Ernte benötigten Arbeitsaufwand zu verringern. Sie besteht darin, daß kurz vor der Ernte eine große Anzahl Proben aufs Geratewohl von den einzelnen Parzellen über eine bestimmte abgemessene Reihenlänge hin genommen werden, die dann gewogen und — wenn es sich um einen Versuch mit Getreide handelt — mit einer Miniaturdreschmaschine ausgedroschen werden. Der Rest des Bestandes bleibt zunächst noch stehen und wird dann in der üblichen Weise geerntet, ohne daß es nötig wäre, irgendwelche Gewichtsbestimmungen vorzunehmen. Dadurch bleibt die ganze Arbeit erspart, die durch getrenntes Ernten, Aufstellen und Dreschen der einzelnen Teilstücke entstehen würde. Obendrein werden Verluste vermieden. Ein Vergleich der neuen und alten Methode hat gezeigt, daß die neue sehr aussichtsreich ist. Vom Standpunkt des Chemikers aus aber hat sie den Vorzug, ausgezeichnete Proben zu Analysenzwecken zu liefern.

Die Erhöhung der Sicherheit von Feldversuchsergebnissen rechtfertigt die Ausdehnung derartiger Untersuchungen über die Beziehungen zwischen Ertrag und denjenigen Witterungs- bzw. Bodenfaktoren, die zahlenmäßig ausgedrückt werden können.

Mit der Entwicklung der Feldversuchstechnik hat sich auch das Feldversuchswesen seit 1919 insofern entwickelt, als Arbeitsgemeinschaften ins Leben gerufen worden sind, deren Aufgabe es ist, Beobachtungen während der Vegetationszeit auf dem Felde anzustellen und die Ergebnisse der Versuche zu verarbeiten. Eine derartige Gruppe oder Arbeitsgemeinschaft setzt sich zusammen aus einem vorgebildeten Landwirt, einem Pflanzenphysiologen, einem Ökologen, einem Chemiker und einem Statistiker. Auf den Versuchsfeldern werden provisorische Laboratorien errichtet, um die nötigen Messungen und Beobachtungen über die Zuwachsgeschwindigkeit bei Getreide, das Auszählen der Bestockung, Feststellung des Ährenschiebens, der Stroh- und Ährenanlage sowie der Anzahl Körner je Ähre bequemer vornehmen zu können. Bei Rüben und Kartoffeln werden neben der Größe der Pflanze auch ihre seitliche Ausbreitung und die Eigentümlichkeiten der Blattentwicklung gemessen usw. Diese Beobachtungen versprechen zur Klärung der Frage nach dem Einfluß des Bodens, des Klimas und der Düngung auf den Pflanzenertrag einmal bedeutungsvoll zu werden.

Viele der verwickelten Fragen, die sich aus gewöhnlichen Feldbeobachtungen ergeben, werden auf diese Weise zu Fragen der Pflanzenphysiologie vereinfacht, die dann mit den präzisen Laboratoriumsmethoden bearbeitet werden können. Eine enge Zusammenarbeit mit der pflanzenphysiologischen Abteilung des Imperial College unter Professor *V. H. Blackman* hat sich daher entwickelt. Von dort aus werden alljährlich Hilfskräfte zu uns herausgeschickt.

Die Bedeutung der organischen Substanz für die Bodenfruchtbarkeit.

Die Mikroorganismen des Bodens.

In all den alten Rothamsteder Feldversuchen war Stallmist als Vergleich den „künstlichen“ Düngemitteln gegenübergestellt worden, und es hatte sich gezeigt, daß er infolge Verrottung Nährstoffe für die Pflanzen lieferte. Über den Verlauf dieser Verrottung waren dagegen von *Lawes* und *Gilbert* selbst keinerlei Untersuchungen gemacht worden. Im ganzen ist diese Frage 4mal in Rothamsted in Angriff genommen worden: Das erstemal zu ihren Lebzeiten durch *Robert Warington* im Jahre 1878, die übrigen dreimal später.

Ungefähr bis zum Jahre 1835 hatte man gemäß *Liebigs* Lehre gemeint, Ammoniak sei die eigentliche Form, in der die Pflanze den Stickstoff aufnimmt. Die französischen Chemiker wiesen dann nach, daß allenthalben Nitrate im Boden vorkommen, und erklärten demzufolge, die Nitrate seien die eigentliche Nährstofform für Pflanzen. Sie erinnerten an die altbekannte praktische Erfahrung, die man mit Salpeterbeeten gemacht hatte, und betonten die Bedeutung der Nitrifikation im Boden. 1877 machten dann *Schloesing* und *Müntz* die glänzende Entdeckung, daß Mikroorganismen die Nitratbildung verrichten. Unmittelbar danach begann *Warington* in Rothamsted mit dem Studium des Vorganges und zeigte, daß er in 2 Stufen verläuft: Ammoniak wird in Nitrit, Nitrit in Nitrat umgewandelt. Er machte wiederholt den Versuch, die Organismen zu entdecken, hatte aber, da sich stets Peptone oder Gelatine in seinen Kulturmedien befanden, keinen Erfolg, obwohl seine Technik für die damaligen Verhältnisse vorzüglich war. Seine Forschungen zeigten aber immerhin deutlich, welch ungeheure Bedeutung den Bakterien bezüglich der Ertragsfähigkeit des Bodens zukommt.

Zum zweiten Male wurde das Thema in Angriff genommen vor etwa 25 Jahren: Der Verfasser dieser Arbeit hatte nämlich beobachtet, daß in fruchtbaren Böden die Oxydation rascher verläuft als in unfruchtbaren. Da die Oxydation im Zusammenhang steht mit der Aktivität der Mikroorganismen, so folgt daraus, daß die Tätigkeit der kleinsten Lebewesen in fruchtbaren Böden reger ist als in unfruchtbaren. Steigerung der Lebensfähigkeit der Mikroorganismen, z. B. durch teilweise Sterilisation des Bodens, erhöhten auch den Grad der Bodenfruchtbarkeit. War die Steigerung der Aktivität jedoch durch Verabfolgung nicht N-haltiger Nährstoffe wie Zucker Stärke usw. bewirkt worden, so nahm die Bodenfruchtbarkeit ab. Im Verfolg der wissenschaftlichen Untersuchung dieser Fragen stellte es sich heraus, daß die Bodenpopulation sehr komplex ist und eingehendere Untersuchungen ihrer wichtigsten Vertreter erforderlich sind. In einem späteren Abschnitt werden die bisherigen Ergebnisse zur Besprechung kommen.

Für praktische Zwecke wurden Methoden zur teilweisen Sterilisierung des Bodens ausgearbeitet. Für Tomaten- und Gurkenanbauer sind diese Methoden sehr wertvoll. Sie haben deshalb in den Gewächshauskulturen Englands in großem Stil Anwendung gefunden.

Übersicht 28. *Teilweise Sterilisierung des Bodens zum Anbau von Tomaten in Handelsgärtnereien.*

		Ertrag an Tomaten dz/ha
A.	Nicht sterilisierter Boden	553
	Nach Behandlung mit Dampf	1045
B.	Nicht sterilisierter Boden	779
	Nach Behandlung mit Cresybicsäure	955

(10 dz Tomaten kosten etwa 820,— RM.)

Die Wirkungen der teilweisen Sterilisation sind sehr verwickelt. Diese hat nicht nur biologische, sondern auch chemisch-physikalische Veränderungen im Boden zur Folge.

Die 3. Gruppe von Untersuchungen wurde im Jahre 1914 mit einer Arbeit *E. H. Richards* und des Verfassers Arbeit über die Zersetzung des Stallmistes (besonders während der Lagerung) eingeleitet. Es stellte sich heraus, daß bei der Zersetzung von Stroh Ammoniak in eine unlösliche Stickstoffverbindung übergeführt wird, vermutlich ein Assimilationsprodukt der Mikroorganismen, wie schon früher *Maerker* und *Schneidewind* bei ihren Untersuchungen über Stallmistbildung angenommen hatten. *E. H. Richards* und *H. B. Hutchinson* studierten diese Strohzersetzung in ihren Einzelheiten und fanden, daß der Prozeß dahin beeinflußt werden kann, daß er ein dem Stallmist ähnliches Endprodukt liefert. Die Grundbedingungen dafür sind, daß Luft, Wasser, Stickstoff, Phosphorsäure u. a. Nährstoffe sowie Calciumcarbonat in genügender Menge vorhanden sein müssen. Außerdem muß das Substrat säurefrei sein. Die Organismen brauchen nie besonders hinzugefügt zu werden, denn sie sind immer am Stroh oder anderen Pflanzenabfällen schon vorhanden. Die Untersuchung zerfiel in 2 Abschnitte: die praktische Seite, zu entdecken, wie verschiedenerlei Pflanzenabfälle am besten in wirksamen Humusdünger verwandelt werden können, und die wissenschaftliche Seite, die darin besteht, den eigentlichen Verlauf des Rottevorgangs eingehender zu studieren. Der praktische Teil fand großen Anklang bei den Gärtnern Großbritanniens. Sie brauchen große Mengen Stallmist, ohne Gelegenheit zu haben, ihn selbst herzustellen oder zu kaufen. Natürlich hat das neue Verfahren auch großes Interesse für den Landwirt, der Weizen und Mais baut, den Pflanzer in den Zuckerrohrgebieten und alle diejenigen, in deren Betrieben große Mengen Stroh und andere pflanzliche Abfallprodukte anfallen, die als Viehfutter nicht geeignet sind und deshalb nicht auf diese Weise in Stallmist umgewandelt werden können. Die Anfragen bei uns häuften sich bald so, daß wir nicht mehr allein damit fertig wurden. Wir legten die Durchführung der praktischen Seite daher in die Hände einer Gesellschaft, die den Namen „Adco“ trägt. Alle Gewinne über $5^1/_2$ % des Anlagekapitals führt diese Gesellschaft in einen Fond zur Förderung wissenschaftlicher

Untersuchungen ab. Diese Lösung wird als sehr brauchbar empfunden. Als ein Beispiel für die Arbeit, die „Adco“ im Empire leistet, möchte ich folgende Versuche anführen, die an der Versuchsstation Salisbury in Süd-Rhodesien ausgeführt worden sind:

Übersicht 29. *Adco-Düngungsversuch an der landwirtschaftlichen Versuchsstation Salisbury, Süd-Rhodesien.* 1925—1926. Kartoffeln, Sorte Up to date.

	Ertrag dz/ha
Kraalmist	455,1
Adco-Kunstdung aus mexikanischer Studentenblume (Tagetes)	428,7
„ „ frischem Gras	404,5
„ „ Maisabfall	393,6
„ „ dürrem Gras	336,2
Ungedüngt	158,7

Das Adco-Verfahren ist jetzt stark verbreitet, Tausende von Tonnen künstlichen Stallmistes werden alljährlich mit Hilfe des Adco-Pulvers hergestellt. Interessant ist der Verlauf der Zersetzung. 2 Bedingungen müssen erfüllt sein:

1. Genügend Stickstoff und Phosphorsäure müssen vorhanden sein. Weizen- oder Gerstenstroh enthalten nicht genug Stickstoff. Es müssen daher 0,7 Teile N auf 100 Teile trockenes Stroh zugesetzt werden. Die Verrottung geht dann so weit, bis etwa 50 % der Trockensubstanz verschwunden sind. Dabei treten keinerlei Stickstoffverluste ein: 2 % N ist der Gehalt des Endproduktes. Werden dagegen mehr als 0,7 % N zum Ausgangsmaterial hinzugefügt, so kann der Überschuß verlorengehen.

2. Es darf nicht zu viel Lignin im Verhältnis zu den anderen nicht N-haltigen Substanzen (Hemicellulosen, Cellulosen, Xylanen) im Ausgangsmaterial enthalten sein. Die letztgenannten dienen den Organismen zur Energiequelle. Das Lignin wird dagegen nicht von ihnen angegriffen. Material, das mehr als 20 % Lignin oder weniger als 3 Teile Cellulose (bestimmt nach der Methode von *Cross* und *Beaven*) auf 1 Teil Cellulose enthält, zersetzt sich nur langsam.

Rege[1] und *Norman*[2] haben etwas festere Grenzwerte angegeben für die Bestimmung, für die sie geeignete Methoden ausgearbeitet haben:

Übersicht 30. *Humusbildung infolge Verrottung von Pflanzenmaterial.*

	Cellulose	Lignin	Reges Verhältnis	Normans Verhältnis	Verlauf des Rottevorganges
Reisstroh	45,9	10,3	1,54	6,3	Rasch
Haferstroh	51,7	14,2	1,13	5,0	„
Gerstenstroh	49,4	16,2	1,06	4,3	„
Weizenstroh	52,1	14,6	1,04	4,9	„
Schilf	51,3	17,7	0,86	3,9	Langsam
Maisstroh	45,0	20,3	0,75	3,0	„
Eschenzweige	53,4	28,4	0,43	2,6	Sehr langsam
Pappelzweige	66,3	28,4	0,34	2,7	Desgl.
Kiefernzweige	57,4	26,7	0,12	2,3	Gar nicht

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[1] *R. D. Rege*, Ann. of Applied Biology **14**, 1.

[2] *A. G. Norman*, Biochem. J. **23**, 138 (1929).

Die Arbeiten über den chemischen Ablauf des Prozesses werden weitergeführt. Zuerst werden von den Bestandteilen des der Rotte unterworfenen Strohes die Hemicellulosen und die Cellulosen zersetzt, sofern sie nicht von einer allzu widerstandsfähigen Ligninschicht inkrustiert sind. Die Hemicellulosen werden aber nur zum Teil abgebaut. Wenn die Cellulosen verrottet sind, wird das sie umschließende oder an sie anlagernde Xylan frei und ebenfalls abgebaut. Das Pektin zerfällt dagegen nicht oder nur dann, wenn das Substrat angesäuert wird. Vom Lignin bleibt, wenn auch nicht alles, so doch der größte Teil unzersetzt. Quantitativ ist der Rottevorgang nach Untersuchungen von *Norman* in Abb. 9 dargestellt.

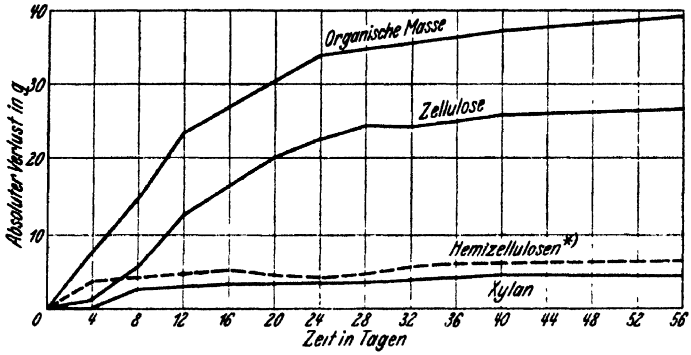


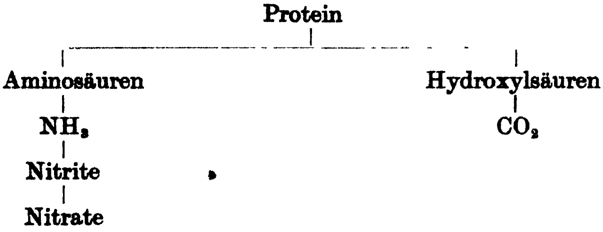
Abb. 9.

* Bestimmt als Furfuraldehyde ihrer Pentosane.

Die Organismen, die im Rotteprozeß eine Rolle spielen, sind vorwiegend Pilze. Unter ihnen befinden sich verschiedene Arten von Aspergillus (fumigatus, nidulans, niger, terreus), verschiedene Actinomyceten, ein Trikoderma und ein thermophiler Organismus: Sepedonium. Diese sind alle erst bei verhältnismäßig hohen Temperaturen aktiv, der letztgenannte hat sein Optimum bei 45—50° und hält selbst Wärmegrade von über 60° aus, wie sie im allgemeinen in der Natur nicht vorkommen. Haben doch die meisten Lebewesen ihr Optimum bei etwa 22° und wachsen schon nicht mehr recht bei Temperaturen von über 35°.

Die genannten Organismen sind also nicht nur wärmeliebend, nein, sie benötigen höhere Temperaturen sogar zu ihrer Entwicklung. Nach Überimpfungen auf steriles Stroh zersetzen sie dies so lebhaft, daß die freiwerdende Wärme die Temperatur rasch bis auf 40° und mehr erhöht. Die Hemizellulosen scheinen in ganz besonderem Maße daran beteiligt zu sein, die notwendigen Energiemengen zu liefern.

Die neueste Entwicklung der Tätigkeit auf diesem Gebiet geht in Rothamsted dahin, alle unsere mikrobiologischen Kenntnisse zu sammeln und sie auf das Studium der im Boden vorgehenden Veränderungen anzuwenden. Die modernen Untersuchungen haben die Ergebnisse der älteren Forschung insofern bestätigt, als sie zeigen, daß die Zersetzung frischer organischer Masse im Boden sich gleichzeitig nach 2 Richtungen vollzieht: Protein und andere Stickstoffverbindungen zersetzen sich unter Bildung von Ammoniak, aus dem dann Nitrite und als nächste Stufe Nitrate aufgebaut werden:



Unsere interessanteste Entdeckung der letzten Jahre ist es, daß die Oxydation von Ammoniak zu Nitrit nicht beschränkt ist, wie man bisher annahm, auf Nitrosomonas und Nitrococcus. Sie wird vielmehr durch eine ganze Reihe anderer Organismen verursacht, von denen viele besonders bei Gegenwart leicht zersetzbarer organischer Masse gedeihen.

Die Hauptuntersuchungen betreffen aber die Umwandlung von nichtstickstoffhaltigen Bestandteilen im Boden. Nach den bisherigen Erfahrungen zu urteilen, scheint bezüglich der chemischen Veränderungen weitgehende Ähnlichkeit mit den Verhältnissen beim Rottevorgang des Stallmistes auf der Düngerstatte zu bestehen, die schon besprochen worden sind.

Das charakteristischste Produkt der Verrottung ist jene schwarze kolloidale Substanz, die unter dem Namen Humus allgemein bekannt und eingehend untersucht worden ist. *Du Toit* hat in unserem Laboratorium gezeigt, daß enge Beziehungen bestehen zwischen gebildeter Humusmenge und der Menge der zersetzten Cellulose und des Lignins. Die meisten Forscher sind sich heute darin einig, daß der Humus aus einem dieser Stoffe allein oder aus beiden gebildet werden kann, daß die Hauptbildungsquelle aber das Lignin darstellt. Es sind verschiedene Reaktionen möglich: *Maillard* zeigte, daß durch Kondensation von Zuckerarten bei Anwesenheit von Aminosäuren etwas dem Humus sehr Ähnliches entsteht. Dies würde für Humusbildung aus Cellulose sprechen. *Fischer* und *Schröder* fanden, daß Lignin unter alkalischen Bedingungen leicht Sauerstoff absorbiert, wodurch eine schwarze Substanz gebildet wird, die dem Humus sehr ähnlich ist. Andererseits zeigte *Waksman*, daß ein Gemisch von Pilzmycel und Lignin ebenfalls dem Humus sehr ähnelt. Wahrscheinlich kommen unter natürlichen Bedingungen alle 3 Entstehungsweisen nebeneinander vor.

In Düngerhaufen wird die Rotte, wie schon erwähnt, durch Pilze verursacht. Im Boden bestehen viel mehr Möglichkeiten. Wenigstens

3 Gruppen von Bodenorganismen sind befähigt, Cellulose zu zersetzen: Pilze, einschließlich der Actinomyceten, Spirochäten und Bakterien. Die Bodenreaktion gibt den Ausschlag dafür, welche der 3 Gruppen überwiegt. In sauren Böden (p_H 4—5) sind die Pilze und Actinomyceten am aktivsten, auf jeden Fall vermehren sie sich lebhaft, wenn Cellulose dem Boden einverleibt wird. In weniger sauren Böden (p_H 5,4—6,6) spielt Spirochaeta cytophaga die Hauptrolle; sie vermehrt sich ganz außerordentlich lebhaft. Unter neutralen Bodenverhältnissen scheinen die kurzen, stäbchenförmigen, Cellulose zerstörenden Bakterien hauptsächlich tätig zu sein. Eine Reihe von diesen sind isoliert und untersucht worden von *Kalnins*. Dieser Wechsel entsprechend der Reaktion tritt sowohl unter Rothamsteder Verhältnissen wie in den Tropen ein und scheint unabhängig vom Bodentyp zu sein.

Die beiden Prozesse: Abbau der Stickstoffverbindungen und Zersetzung der Cellulose und anderer Kohlehydrate scheinen nicht nur gleichzeitig zu verlaufen, sondern sogar eng miteinander verknüpft zu sein. Die Zersetzung von Cellulose liefert dem Organismus so viel Energie, daß sie sich rasch vermehren können, solange es nicht an Nährstoffen (vorwiegend Stickstoff und Phosphorsäure) gebricht. Ja, die Cellulosezersetzung und Verrottung von pflanzlichen Abfällen steht quantitativ in enger Beziehung zu dem Vorrat des Bodens an Stickstoff und kann gesteigert werden durch Zusatz von Ammoniumcarbonat oder Harnstoff. Daraus folgt, daß die durch Verrottung organischer Masse anfallende Menge von Nitraten im Boden nicht nur bestimmt wird von der vorhandenen Menge Stickstoff, sondern auch von der Menge leicht zersetzlicher Cellulose und anderer Kohlehydrate. Der kritische Wert scheint etwa bei 1,5 % Stickstoff in der Trockensubstanz zu liegen oder besser bei einem C : N-Verhältnis von 30 : 1 erreicht zu sein. Unseren Erfahrungen nach liefern Pflanzenreste mit weniger Stickstoff keine Nitrate bei ihrer Verrottung, da all ihr Stickstoff zur Ernährung der Organismen verbraucht wird, ja es kann dahin kommen, daß sie den Boden sogar seines Nitratvorrates berauben und dadurch seine Produktionskraft schwächen. Ausgangsmaterial mit einem höheren Stickstoffgehalt hinterläßt einen Überschuß von Nitraten im Boden, der jedoch selten mehr als etwa 40—50 % der ursprünglichen Stickstoffmenge ausmacht. Das hat *H. L. Jensen* in unserem Laboratorium gefunden. Seine Untersuchungsergebnisse im neutralen und saurem Boden sind wiedergegeben in Übersicht 31.

Die quantitativen Beziehungen sind dargestellt in Abb. 10.

Wie auch immer die Zusammensetzung des Ausgangsmaterials gewesen sein mag, das Endprodukt des Rottevorganges hat einen annähernd konstanten N-Gehalt von rund 4 % und einen C-Gehalt von etwa 45 %, ähnlich dem von Pilzmycel. In einem sauren Boden ist der

Übersicht 31. *Zersetzung organischer Masse im Boden.*
(*Nach Jensen.*[1])

Ausgangsmaterial	N %	C : N Verhältnis	% N in Nitrate umgewandelt in neutralem Boden	% N in Nitrate umgewandelt in saurem Boden	N % im gebildeten Boden-Humus [neutraler Boden]
Weizenstroh	0,54	84,0	0	0	3,89
Inkarnatklee	1,74	25,9	13,8	0	4,24
Blaue Lupine	2,26	20,0	17,7	0	4,00
Stallmist	2,33	0	23,6	0	3,85
Erbsen-Schoten	2,90	13,3	40,3	27,6	4,08
Luzerne	3,46	12,9	39,6	0	4,58
Pilzmyzel	4,45	10,2	39,6	5,5	4,97

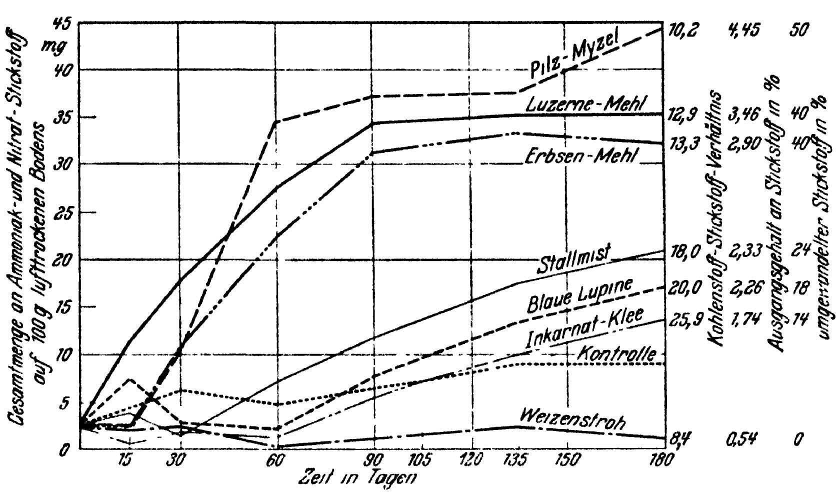


Abb. 10.

Grenzwert des C : N-Verhältnisses viel kleiner, die Organismen brauchen mehr Stickstoff, um die Zersetzung ausführen zu können. Das stimmt überein mit der Anschauung, daß Bakterien mit niedrigem N-Bedarf in neutralen Böden vorherrschen, in sauren dagegen Pilze mit höherem Bedarf an Stickstoff überwiegen.

Diese Befunde stehen in scharfem Gegensatz zu denen, die bezüglich der Nitrifizierung von anorganischen Substanzen wie Harnstoff und Ammoniaksalzen gemacht worden sind. Im letzteren Falle liefert der Nitrifikationsprozeß an Nitraten nahezu 100 % der zugefügten N-Menge. Jetzt sind wir in der Lage, 2 der wichtigsten Unterschiede zwischen organischen und anorganischen Düngemitteln zu erklären: 1. Organische Düngemittel haben geringere Düngewirkung als anorganische wie Ammoniumsalze, Harnstoff usw. 2. Organische Düngemittel können den Stickstoffgehalt des Bodens erhöhen, was anorganische nur selten tun.

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[1] *H. L. Jensen,* J. agricult. Sci. **19**, 71 (1929).

Einige der Rothamsteder Ergebnisse über die Wirksamkeit von Düngemitteln sind wiedergegeben in Übersicht 32.

Übersicht 32. *Wiedergewinn von N in der Ernte in Prozenten der in Form von Düngemitteln aufgewandten Menge.*

	Volldüngung mit			Rapskuchen	Stallmist
	Natronsalpeter	Schwefelsaurem Ammoniak			
Weizen: Korn	26	26,0		15,0	13
Stroh	10	9,0		7,5	5
Gesamt	36	35,0		22,5	18
Futterrüben: Rüben	60	42,5		49,0	28
Blatt	17	12,5		16,0	8
Gesamt	77	55,0		65,0	36
		N dz/ha 0,25	0,5		
Kartoffeln: Knollen	—	49,0	36,0	—	—
Kraut	—	23,5	16,0	—	—
Gesamt	—	72,5	52,0	—	—

Die Stallmistgabe ist ziemlich groß, so daß daraufhin die Ausnutzung nicht so gut wie gewöhnlich sein mag. Zu Weizen sind alle Gaben hoch und die Ausnutzung ist entsprechend gering. Der Einfluß der Düngemittel auf den Stickstoffgehalt des Bodens ist besonders deutlich in den Analysenwerten von den Böden der verschieden gedüngten Teilstücke des Weizenversuches auf Broadbalkfield, die seit 1843 einer gleichförmigen jährlichen Düngung unterworfen sind. Der Stickstoffgehalt des Bodens war 1865 bestimmt worden. Die Bestimmung wurde dann später in Abständen mehrfach wiederholt. Auch die Ernten wurden analysiert. Die Bilanz über die 50 Jahre von 1865—1914 sieht folgendermaßen aus:

Übersicht 33. *Broadbalkfield. Stickstoffbilanz 1895—1914.* (Oberste 23 cm.)

N-Gehalt	Stallmist (Parzelle 2B) kg/ha	Ungedüngt seit 1839 (Parzelle 3) kg/ha	Volldüngungen (Parzelle 7) kg/ha	(Parzelle 13) kg/ha
Des Bodens: 1865	5420	3310	3790	3710
1914	6240	2880	3590	3610
Differenz nach 49 Jahren	+820	—430	—200	—100
Der Düngung (jährlich)	233	8	104	104
Der Ernte (jährlich)	56	19	51	49
Gewinn (+) oder Verlust (—) des Bodens (jährlich)	+17	—9	—4	—2
Unkontrollierbare Diff. (jährlich)	160	(+2)	57	57

Mit der Stallmistgabe werden dem Boden jährlich 224 kg/ha Stickstoff zugeführt. Davon haben im Durchschnitt von 50 Jahren 45 kg jährlich dazu gedient, den Boden an Stickstoff anzureichern, wohingegen mit einer Volldüngung, die 48 kg Stickstoff in Form von schwefelsaurem Ammoniak enthielt, pro Jahr und Hektar nur 1,75 kg im Boden angehäuft worden sind. Der Rotteprozeß verläuft aber derartig, daß in allen 3 Böden selbst nach Verlauf von 50 Jahren die C : N-Verhältnisse annähernd gleich geblieben sind:

Übersicht 34. *C:N-Verhältnis in Böden Broadbalkfields.*

	1881	1893	1922
Stallmist.	11,4	10,6	11,1
Ungedüngt.	9,2	8,8	9,6
Volldüngung	10.8	9.0	9.6

Die Ergebnisse sind aufgeklärt worden durch *Jensens*[1] Versuche, welche zeigen, daß bei organischer Substanz mit weniger als 1,5 % Stickstoff der Stickstoffgehalt nicht ausreicht, den Bedarf der Organismen zu decken, die ihre Zersetzung bewirken. Wenn sie also trotzdem in den Boden gebracht wird, so hat dies zur Folge, daß die im Boden befindlichen Nitrate in Form von Körpereiweiß von den kleinen Lebewesen festgelegt werden. Später kann dies in leicht lösliche Nitratform zurückverwandelt werden. Die unmittelbare Folge einer solchen Zufuhr von organischer Masse mit zu geringem N-Gehalt ist aber Verminderung des Nitratgehaltes des Bodens und Einbuße an Fruchtbarkeit. Erst später stellt sich dann eine Erhöhung des Fruchtbarkeitgrades dadurch ein, daß die Nitrate wieder frei werden. Am deutlichsten wird das, wenn dem Boden Zucker zugeführt wird. Erfolgt die Zufuhr im Frühjahr kurz vor Aussaat der Gerste, so wird der Ertrag dadurch gedrückt, erfolgt sie dagegen im Herbst vor dem Eintreten der Winterregen, die bei uns in Rothamsted alljährlich ganz erhebliche Auswaschverluste für den Stickstoff zu verursachen pflegen — an die 50—60 kg pro Hektar — so erhöht die Zuckerdüngung den Ertrag der im nächsten Frühjahr zur Aussaat kommenden Gerste, weil die ursprünglich in Form von Körpereiweiß aufgespeicherten Nitrate um diese Zeit in solche zurückverwandelt werden. Die Erträge folgen:

Übersicht 35. *Hoosfield. — Einfluß von Zuckerdüngung auf den Gesamtertrag von Gerste.* (Nach *H. B. Hutchinson*[2].)

Parzellennummer	Düngung	Zuckerdüngung					
		im Frühjahr 1907/1909			im Herbst 1910/1911		
		ohne Zucker dz/ha	mit Zucker dz/ha	durch Zuckerdüngung ± dz/ha	ohne Zucker dz/ha	mit Zucker dz/ha	durch Zuckerdüngung ± dz/ha
6	Ungedüngt	15,7	11,9	—3,8	14,6	14,5	—0,1
4	Phosphors. + Kali	31,9	25,9	—6,0	18,6	24,8	+ 6,2

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[1] J. agricult. Sci. **19**, 71 (1929); **21**, 88 (1931).

[2] J. agricult. Sci. **9**, 92 (1919).

Es gibt tatsächlich kein sichereres Mittel, einen Boden vorübergehend unfruchtbar zu machen, als Zucker. Die Wirkungsweise von Getreidestroh ist allerdings von größerem praktischen Interesse. Gewöhnlich hat Stroh einen Stickstoffgehalt von weniger als 1,5 % und verursacht daher eine Festlegung von Nitraten im Boden. Die Folge ist: Geringerer Ertrag der darauffolgenden Ernte. Werden dagegen dem Boden obendrein genügend Nitrate durch eine besondere Düngung zugeführt, so tritt keine Ertragsdrückung ein, ja der Ertrag kann sogar gesteigert werden infolge Freiwerdens von Kali aus dem Humus oder dem Stroh. Einen Beweis dafür liefern die Ergebnisse von Broadbalkfield.

Übersicht 36. *Einfluß einer Strohdüngung auf die Gesamterträge von Weizen und Klee.*

Parzellennummer	Stickstoff kg/ha	Broadbalk 1868—1879		
		ohne Stroh dz/ha	mit Stroh dz/ha	Einfluß der Strohdüngung dz/ha
		Weizen.		
8	Überschuß . . 144	70,1	70,9	Zum Vorteil
7	96	58,1	55,7	Zum Nachteil
6	Ungen. Menge 48	38,1	36,4	Desgl.
		Klee.		
Ohne N		Weniger Knöllchen	Mehr Knöllchen	Zum Vorteil

Leguminosen sind nicht so sehr auf die Nitrate im Boden angewiesen, im Gegenteil, sie bilden sogar ihre Knöllchen besser aus, wenn Nitrate fehlen. So kommt es, daß sie von einer Strohdüngung Vorteil haben können. Allem Anschein nach kommt aber noch etwas anderes hinzu: Ein bestimmter Bestandteil des Strohes ist unmittelbar förderlich für die Knöllchenbakterien. Diese Versuche erklären die den englischen Landwirten wohlbekannte Erfahrungstatsache, daß gut verrotteter Stallmist unbeschadet der künftigen Erträge im Frühjahr gegeben werden kann, während schlechtverrotteter strohiger Mist schon im Herbst untergepflügt werden sollte.

Diese Verhältnisse der Festlegung von Nitraten im Boden sind nun aber günstig für die Bindung freien atmosphärischen Stickstoffs, und es besteht die Aussicht, daß diese dann vor sich geht. Wir in Rothamsted haben allerdings keine sicheren Anhaltspunkte für eine derartige Bindung von Nitratstickstoff aus der Luft durch Mikroorganismen des Bodens, obwohl die in Frage kommenden Organismen überall in unseren Böden vorkommen.

Um all die verschiedenen Fragen mehr im einzelnen zu studieren, ist 1930 auf Hoosfield in Rothamsted ein neuer Versuch angelegt worden, der 20 Jahre hindurch fortgesetzt werden soll. Zweck dieses Versuches ist es, die Wirkung von Stallmist mit der von künstlich zum Verrotten gebrachten Stroh zu vergleichen, ferner mit der Wirkung von Stroh, das zusammen mit Stickstoffdüngemitteln untergepflügt worden ist, um die Verrottung zu beschleunigen, und endlich mit der Wirkung von künstlichen Düngemitteln allein. Es handelt sich um einen Fruchtfolgeversuch mit der Folge: Gerste, Kleegras, Weizen, Kohlrüben. In den Klee ist italienisches Rayegras eingesät, um der Kleemüdigkeit vorzubeugen, die manche Jahre in dem Fruchtfolgeversuch auf Agdellfield völliges Ausbleiben der Kleernte zur Folge gehabt hat. Da das Rayegras eine Wirtspflanze für Oscinis frit, die Fritfliege, ist, wird der Bestand, um der Gefahr vorzubeugen, gleich nach dem ersten Schnitt bzw. nach der Heuernte vor Mitte August untergepflügt.

5 verschiedene Düngungen werden gegeben:

1. Stallmist,

2. Adcokompost, d. i. künstlich mit Adcopulver zur Verrottung gebrachtes Stroh.

3. Stroh ohne vorherige Zersetzung. In diesem Falle werden aber künstliche Düngemittel hinzugefügt.

4. Künstliche Düngung allein (Phosphorsäure in Form von Superphosphat).

5. Ebenso, die Phosphate jedoch in Form von gemahlenem Rohphosphat.

Jede der genannten Feldfrüchte kommt alljährlich zum Anbau. Im nächsten Jahre folgt dann die nächste Rotation. Die gesamte Versuchsfläche ist demzufolge in 4 Schläge eingeteilt, auf denen in jedem Jahre eine der 4 verschiedenen Früchte wächst. Die Schläge sind wieder in je 5 Streifen entsprechend den 5 unterschiedlichen Düngeweisen untergeteilt. Ein Streifen besteht aus 5 Parzellen.

Im Verlauf von 4 Jahren ist eine ganze Folge der angebauten Früchte einmal auf einer Parzelle zum Anbau gekommen, und wenn das 5. Jahr kommt und die Düngung wieder verabfolgt werden muß, so ist es nicht die gleiche Frucht, zu der die Düngung ursprünglich gegeben worden war, sondern die nächste in der Fruchtfolge. Auf jeder Parzelle kehrt die gleiche Frucht jedes 4. Jahr wieder, die gleiche Düngung aber nur jedes 5. Jahr. Gleiche Frucht und gleiche Düngung hat eine Parzelle also nur einmal im Laufe von 20 Jahren.

5 Streifen zu 5 Parzellen ergeben 25 Parzellen für jede Frucht; für alle 4 Früchte sind demnach im ganzen 100 Teilstücke nötig. In den einzelnen Jahren gibt es keine Wiederholungen der verschieden behandelten Teilstücke. Am Ende von 20 Jahren werden die 5 Turnus der 4feldrigen Fruchtfolge in 5facher Wiederholung vorhanden sein und die vier 5jährigen Turnus der Düngeranwendung sind dann 1mal durch die ganze Folge von Früchten hindurch gewandert.

Die Mikroorganismenwelt des Bodens.

Wir kommen nun zu den Bewohnern des Ackerbodens. Mikroorganismen gibt es sehr viele im Boden, und zwar sehr mannigfaltige Arten. Die Anzahl der Arten, die in einem Gramm Boden gefunden werden, bringt Übersicht 37.

Übersicht 37. *Die Mikroorganismen im Ackerboden.*

	Rothamsted.	
	Krume	Untergrund
Bakterien	5000000000	1000000000
Protozoen: Ciliaten	1000	100
Flagellaten. . . .	770000	350000
Amöben.	280000	150000
Algen (nicht blaugrün) . . .	100000	—

Die Anzahl der vorkommenden blaugrünen Algen, Actinomyceten und Nematoden sind nicht bekannt. Die Pilze können nicht gezählt werden, wohl aber kann ihre Mycelmasse mit der Körpermasse der Protozoen verglichen werden. Die vorkommenden Arten scheinen auf der ganzen Welt nahezu die gleichen zu sein. Untersuchungen an Bodenproben, die von verschiedenen Stellen Spitzbergens bis hinunter zum Äquator und von da hinab bis zur Antarktis stammten, haben kein deutliches Bild vom Vorhandensein einer spezifischen geographischen Verteilung gegeben, wenn man von einigen wenigen Spezies absieht.

Verschiedene Fragen bezüglich der Eigenarten der Bodenpopulation gehen jetzt ihrer Lösung entgegen. Eine der wichtigsten Entdeckungen ist es, daß die Anzahl der Organismen nicht konstant ist. *H. G. Thornton* konnte nachweisen, daß die Anzahl der Bakterien stündlich schwankt. *D. W. Cutler* fand, daß die Protozoen zahlenmäßig sich innerhalb eines Tages verändern (sie in Abständen von 1 Std. zu zählen gelingt vorläufig noch nicht). Ihre Anzahl ist umgekehrt proportional der der Bakterien. Andere Versuche ergaben, daß sich die Protozoen von Bakterien nähren und daß sie dabei eine ganz bestimmte Auswahl unter den Bakterienarten treffen. Bestimmte Bakterien haben einen höheren Futterwert für sie bzw. eine höhere Reproduktionskraft als andere. Unterdrückung der Protozoen hat Vermehrung der Bakterien zur Folge. Es treten aber in den verschiedenen Jahreszeiten bei allen Bodenorganismen gleichlaufende Schwankungen ihrer Anzahl auf, soweit Zählungen vorgenommen worden sind. Im Frühjahr besteht allgemein die Tendenz zu stärkerer Vermehrung, im Sommer nimmt die Zahl wieder ab, um im Herbst abermals anzusteigen. Im Winter ist sie dann wieder niedrig.

Ziemlich viel ist in unseren Laboratorien darüber gearbeitet worden festzustellen, welche Ursachen diesen zahlenmäßigen Schwankungen zugrunde liegen. Es bestehen keine unmittelbaren Beziehungen zur Temperatur oder zur Wasserzufuhr. Vielmehr dürfte diese Erscheinung zum Teil mit der Art der Vermehrung der Organismen im Zusammenhang stehen.

Die meisten der in Frage kommenden Kleinlebewesen haben, soweit sie darauf untersucht sind, einen ziemlich komplizierten Entwicklungscyclus zu durchlaufen, innerhalb dessen nur 1 oder 2 Zeitabschnitte bestehen, während deren eine Vermehrung möglich ist. Alle Organismen brauchen in gleicher Weise Zufuhr von Nährstoffen und Energie, und alle scheinen unter gewöhnlichen Verhältnissen sich ungehemmt bis zu einer maximalen Höchstgrenze zu vermehren. Jede Zufuhr von Energie oder Nährstoffen hat unmittelbare Vergrößerung ihrer Anzahl zur Folge, solange es die übrigen Bedingungen gestatten. Die umgesetzten Energiemengen sind oft gewaltig groß. Auf den Weizenparzellen Broadbalkfields, von denen schon vorher die Rede war, finden schätzungsweise alljährlich folgende Energieumsetzungen statt:

Übersicht 38. *Broadbalkfield. Jährliche Energieumsetzungen im Boden*
Millionen Kilogramm Calorien je ha und Jahr.

	Stallmist	Ungedüngt
Mit der Düngung zugeführt	35	—
In der Stoppel enthalten	5	0,7
Summe	40	0,7
Dem Boden entzogen	—	1,2—2,0
Im Boden angehäuft	1,2—2,0	—
Verflüchtigt im Jahr	38	2
Berechnet auf den Tag in Cal.	101200	6700
Ausreichend, um den Energiebedarf zu decken, v.	30 Mann	2 Mann
Die von Menschen angebauten Pflanzen liefern ausreichende Energiemengen für	5 „	1 „

Die Bodenorganismen scheinen demnach dem Boden mehr Energie entnehmen zu können als die Menschen.

Es besteht ein gewaltiger Unterschied zwischen Energie und Nährstoffen: Die Nährstoffe können immer wieder benutzt werden, die Energie nicht. Jede Steigerung der Anzahl einer Gruppe von Organismen bedeutet notwendigerweise den Tod für andere, sofern nicht die Energiezufuhr in gleichem Maße vermehrt wird. Bakterien scheinen eine ganz besonders große Fähigkeit zu besitzen, Zersetzungen einzuleiten, um sich mit Nahrung und Energie zu versorgen. Bei der Proteinzersetzung bilden sie größere Mengen von Ammoniak als Pilze und andere Organismen.

In sauren Böden ist die Anzahl von Mikroorganismen bei weitem eingeschränkter, denn viele von den Organismen sind empfindlich gegen Säure, besonders Bakterien und größere Tiere wie Erdwürmer und Schnecken. Pilze und Protozoen werden dagegen weniger beeinträchtigt.

Eine zahlenmäßig große Population ist verhältnismäßig weniger wirkungsvoll als eine kleine, und im allgemeinen sind die Bakterien wenigstens lebhafter tätig, wenn sie mit anderen Arten von Organismen vergesellschaftet sind, als wenn sie allein auftreten. Symbiose scheint allgemein verbreitet zu sein.

Eingehende Untersuchungen wurden mit einigen besonderen Spezies gemacht, namentlich mit den Knöllchenorganismen. *H. G. Thornton*[1] bearbeitet dieses Gebiet. Er fand, daß diese Organismen einen Lebenscyclus durchlaufen und nur auf einigen Stufen dieses Cyclus bewegliche Formen ausbilden, die allein fähig sind, die Infektion der Pflanzenwurzel zu bewerkstelligen. Die Metamorphose von der nichtbeweglichen zur beweglichen Form kann beschleunigt werden durch kleine Phosphorsäuregaben oder Hinzufügen von etwas Milch. In dieser beweglichen Form vermögen sie die Wurzel einer geeigneten Leguminosenart anzugreifen und in sie einzudringen. Für andere Leguminosenarten besitzen sie keine Virulenz. Die Infektion wird erst möglich, wenn die jungen Pflanzen das erste Paar echter Blätter gebildet haben. Sobald dies der Fall ist, dringt der Organismus ein und die Knöllchen werden sichtbar. Anscheinend wird das Entfalten der jungen Blätter von der Ausscheidung eines Stoffes durch die Wurzel begleitet, der auf den Organismus eine stimulierende Wirkung ausubt. Jedenfalls wird das Wachstum der Knöllchenbakterien auf Agar gefördert, wenn man wässerigen Extrakt von Sandkulturen zusetzt. *Thornton* konnte bisher noch nicht feststellen, um welchen Stoff es sich dabei handelt. Er fand aber, daß der fragliche Stoff nicht in den Blättern gebildet wird, da Entfernung der Blätter unmittelbar nach ihrem Erscheinen keinen Einfluß auf die Knöllchenbildung hat. Der Stoff halt sich lange genug im Boden, um die Bakterien zu befähigen, andere Pflanzen anzugreifen, bei denen die ersten Laubblätter noch nicht entwickelt sind. Wenn daher ganz junge Keimpflanzen, die ihre ersten Blättchen noch nicht entfaltet haben, im Gemisch mit älteren Pflanzen wachsen, so setzt bei ihnen die Knöllchenbildung schon früher ein.

Zwischen der Anzahl der gebildeten Knöllchen und der Anzahl der Organismen im Boden besteht zwar eine feste Beziehung, aber nicht etwa Proportionalität. Vielmehr ist es so, daß immer nur ein kleiner

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[1] *H. G. Thornton* and *N. E. Gangulee*, Proc. roy. Soc., Series B, 99, 427 (1926).— *H. G. Thornton*, Proc. roy. Soc., Series B, 104, 481 (1929). — J. agricult. Sci. 19, 48 u. 373.

Bruchteil der Wurzelhärchen infiziert wird, wie groß auch immer die Anzahl der Bakterien ist. Bei Luzerne, auf Agar gezogen, sind es nicht mehr als 4 %.

Thornton hat diese wissenschaftlichen Erfahrungen praktisch auf die Impfung der Luzerne angewandt. Diese Pflanze wurde im 17. Jahrhundert von Flandern nach England gebracht und ist seitdem in England angebaut worden, allerdings nur in den Grafschaften des Ostens und Südwestens. Auf den Westen oder Süden des Landes hat sich ihr Anbau nicht ausgedehnt, da hier die Erträge zu niedrig und die Ertragssicherheit zu gering ist. Es hat sich nun herausgestellt, daß die Knöllchenbakterien nur in diesen östlichen Anbaugebieten vorkommen. In dem Augenblick aber, in dem diese auch nach dem Westen und Norden gebracht wurden, stiegen die Erträge und die Ertragssicherheit wurde erhöht. *Thornton* arbeitete dann ein Verfahren aus zur Herstellung von Bakterienpräparaten und Überimpfung von Kulturen. Im wesentlichen besteht dies darin, daß diese beim Eintreffen auf dem Hofe vom Landwirt mit Milch und phosphorsaurem Kalk untermischt werden, um möglichst maximale Entwicklung der beweglichen Form aus der unbeweglichen des Präparates sicherzustellen. Die Flüssigkeit wird dann mit dem Saatgut in Berührung gebracht.

Übersicht 39. *Ergebnisse von Luzerne-Impfungen in West- und Nordengland.* (Nach *Thornton*[1].)

Versuchsansteller	Ertrag		N-Gehalt der Trockensubstanz	
	geimpft	ungeimpft	geimpft	ungeimpft
	dz/ha	dz/ha	%	%
Col. E. P. Brassey, Gloucester	137,3	40,8	2,53	2,1
Rt. Hon. Lord Clinton Devonshire	66,6	42,7	3,91	3,13
Clarke and Sons, Somerset	102,7	83,9	4,13	4,14
G. H. Johnstone, Cornwall	77,2	52,6	3,4	3,3
Pennell and Sons, Lincoln	190,3	157,6	2,64	2,56
W. R. Strickland, Yorkshire	125,6	78,0	3,4	2,2

Mit diesem Verfahren sind sehr gute Erfolge erzielt worden. Wo Luzerne vorher überhaupt nicht fortgekommen war, wurden mit einem Schlage gute Ernten gemacht. Die Verbesserung der Erträge betraf aber nicht allein die Steigerung der Erntemasse, sondern auch der Eiweißgehalt wurde erhöht. Die Nachfrage nach Bakterienpräparaten wuchs so sehr, daß wir nicht mehr imstande waren, den Bedarf selbst zu decken. Wir haben daher Abmachungen mit der bekannten biochemischen Firma Messrs. Allen and Hanbury, Bethnal Green, London E 2, getroffen, welchen zufolge die Firma Knöllchenbakterienpräparate unter Rothamsteder Kontrolle herstellt, die dann an die Landwirte abgegeben werden. Impfmaterial für 1 ha Land kostet 7,50 RM. Dieses Abkommen erspart uns ziemlich viel Mühe und hat sich zur größten Zufriedenheit ausgewirkt. Die Nachfrage ist noch im Steigen begriffen. *Thornton* hat auch ein Verfahren ausgearbeitet, das gestattet, die Bakterienkulturen über weite Entfernungen hin zu verschicken. So kamen bei Versand nach Westaustralien die Kulturen in gutem Zustande an, und es wurden die besten Erfahrungen mit ihnen bezüglich Steigerung der Luzerneerträge gemacht, wie aus folgenden Zahlen in Übersicht 39 auf Seite 67 hervorgehen dürfte:

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[1] *H. G. Thornton*, J. Farmers Club **1931**, 19.

Laufende Untersuchungen in Rothamsted.

Wir können nun die in Rothamsted bearbeiteten Einzelfragen über das Wesen der Bodenfruchtbarkeit kurz folgendermaßen zusammenfassen:

1. Die drei am eingehendsten untersuchten Teilfragen sind die der Nährstoffzufuhr, der Versorgung mit Luft und Wasser und die der Bodenreaktion.

2. Steigerung eines der Pflanze zugeführten Nährstoffes pflegt erhöhte Aufnahme des Nährstoffes durch die Pflanze zur Folge zu haben. Für gewöhnlich ist damit auch eine Steigerung der Wachstumstätigkeit der Pflanze verbunden, die allerdings nicht immer im gleichen Verhältnis erfolgen muß, woraufhin dann eine Veränderung der Zusammensetzung der Pflanze eintritt. Von großer Wichtigkeit sind die beiden Verhältnisse C:N und N:K.

3. Diese Veränderung in der Zusammensetzung hat ihrerseits eine Veränderung des Wachstumsrhythmus der Pflanze und ihrer Reaktionsweise gegenüber den Außenbedingungen zur Folge. So kann durch geeignete Düngeweise innerhalb gewisser Grenzen

a) die Widerstandsfähigkeit der Pflanze gegenüber Krankheiten,

b) ihr Verhalten gegenüber verschiedenen klimatischen Verhältnissen beeinflußt werden. Aus beiden lassen sich praktische Nutzanwendungen ziehen.

4. Veränderungen in der Zusammensetzung haben einen Einfluß auf den Marktwert der Ernte. Stickstoffzufuhr beeinflußt besonders den prozentualen Zuckergehalt der Rüben und den Stärkegehalt des Getreidekornes. Dies beeinträchtigt den Wert von Marktware bei

1. Braugerste, indem die Malzausbeute vermindert wird,
2. Zuckerrüben, indem der prozentuale Zuckergehalt sinkt,
3. Kartoffeln, indem die Kochqualität der Knollen leidet.

5. Großen Erfolg verspricht Verwandlung von verhältnismäßig billigen Handelsdüngemitteln in höher im Preise stehende Futtermittel. Der Stickstoff der Düngung wird zum Teil von der Pflanze aufgenommen und in eiweißreiches Viehfutter umgesetzt. Unter englischen Verhältnissen ist dieser Umwandlungsprozeß rentabel.

6. Die Untersuchung über die Mikroorganismen des Bodens geben Aufschluß über die Lebensgeschichte der Bodenpopulation, aus dem nach 4 Richtungen hin praktische Nutzanwendungen gezogen werden können:

1. teilweise Sterilisation des Bodens für Gewächshauskultur,
2. Herstellung von Humusdünger aus Stroh und anderen Abfällen,
3. Herstellung von Düngemitteln aus Fäkalien,
4. Impfung von Luzerne.

Weitere Möglichkeiten sind zum Teil noch in Bearbeitung.

7. Die Untersuchung über physikalische Eigenschaften des Ackerbodens verschaffen uns eine bessere Einsicht in das Wesen der Bodenbearbeitung. Was bisher mehr ein Kunststück genannt werden konnte, wird dadurch zu einer Wissenschaft erhoben.

Es kommt freilich bei all diesen Untersuchungen in erster Linie darauf an, die theoretischen Grundlagen klar zu stellen, das Prinzipielle zu abstrahieren und Gesetzmäßigkeiten aufzudecken. Erst wenn diese Seite sicher fundiert sein wird, können sichere Nutzanwendungen daraus gezogen werden. Ohne dieses theoretische Fundament können wir den Landwirten keinerlei Hilfestellung geben, wie sie es mit Recht von uns erwarten.

8. Die modernen statistischen Methoden zur Analyse von Feld- und Wetterbeobachtungen sowie die modernen Methoden der Feldversuchstechnik setzen uns in Stand, eingehende Untersuchungen über den Einfluß der Witterung auf die Wirksamkeit der Düngemittel und der Bodenbearbeitung anzustellen. Dadurch wird Zergliederung der Wirkungsweise dieser Faktoren auf den Ernteertrag bis ins einzelne ermöglicht. Es ergeben sich somit Aussichten auf Begründung von Versicherungen gegen Wetterrisiko, was eine ganz außerordentliche Erleichterung der Wirtschaftsführung für den Landwirt bedeuten würde.

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PYRETHRUM FLOWERS

A QUANTITATIVE STUDY OF THEIR DEVELOPMENT

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(With Plate XLIV and 9 Text-figures.)

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INTRODUCTION.

UNTIL the researches of Staudinger and Ruzicka (7) had demonstrated the constitution of the active principles of pyrethrum (*Chrysanthemum cinerariaefolium*) there was no method except that of direct biological trial that could be regarded as satisfactory for evaluating the flowers; and although biological methods have in recent years been brought to a moderately high degree of exactitude, they require for accuracy a knowledge of a rather complicated technique. In the past rule-of-thumb methods of evaluating, having little to justify them except tradition and rapidity, have been practised. Chemical methods of analysing pyrethrum which have been recently worked out, although, no doubt capable of further improvement, do represent an advance in the means of evaluation previously available and have given results commensurate with the toxicities obtained by biological methods.

The present investigation was undertaken to follow the development of the flowers of pyrethrum and to ascertain, if possible, the way in which the pyrethrin content varies with the degree of development of the flowers and from plant to plant grown from the same seed in the same soil. The two main objects are, in practice, to some extent conflicting in the sense that for genetical study the larger the variations the more suitable in general the material for selection, whereas, the greater the heterogeneity the less valid the conclusions arrived at with respect to the correlations between the amounts of the active principles and the degree of maturity of the plant. The amount of material required for the analyses has an important bearing on both these aspects, since if large quantities are required it is only possible in exceptional cases, where the yield of flowers is high, to ascertain the degree of variation between the flower heads of one plant, and also since it may, in the early stages of development, render necessary the employment of the product of more than one plant and thus make difficult or impossible a free statistical examination of the data.

It has been the practice in the past to describe and differentiate between samples of pyrethrum flowers in terms of the degree of openness of the flower head, open, half-open, and closed buds being the categories into which the samples were separated. There does not, however, seem to have been any systematic attempt to define these categories, and many people are still in doubt as to whether these ascriptions refer to flowers immediately after harvesting or after being subjected to drying. For many years a superior value was attached to certain of these categories, and the fully open flowers were regarded as inferior. It becomes, in the first instance, a matter of importance to have a precise definition of what is meant by these terms, as, in the trade, they seem to be based upon the external characteristics in dried and withered flowers. We have made some attempts by photographing heads immediately after harvesting and again when completely air dry to ascertain how the external appearances have changed in the process of drying. In the work outlined here the categories of maturity refer only to fresh flowers, as it was noted that the external character of the dry and withered flower heads depended upon small factors which, as yet, we have not been able to specify accurately.

EXPERIMENTAL.

In the grounds of the Plant Pathology Laboratory of the Ministry of Agriculture, Harpenden[1], a bed running north and south, 27 by 9½ ft. was planted with 108 pyrethrum seedlings on July 13th, 1928. The history of the bed as far as recorded is as follows:

Dec. 1926. Moderate dressing of farmyard manure.

April 1927. Potatoes planted.

Oct. 1927. Potatoes dug.

July 4th, 1928. Plot forked over and two barrow loads of coal ashes passing ¼ in. sieve and two bushels of slaked lime added.

25½ ft.

	1 *.E .B .D .C* *.F .G .A .H*	2 *.A .F .D .G* *.B .H .C .E*	3 *.B .G .F .H* *.D .E .C .A*	4 *.C .A .H .E* *.D .F .B .G*	
S 9½ ft.	5 *.F .A .G .C* *.H .D .B .E*	6 *.C .A .G .D* *.F .H .B .E*	7 *.F .G .E .A* *.H .D .B .C*	8 *.F .C .E .B* *.A .G .D .H*	N
	9 *.B .G .H .D* *.C .F .E .A*	10 *.B .D .G .A* *.C .H .E .F*	11 *.D .G .H .C* *.A .E .F .B*	12 *.G .B .D .A* *.C .E .H .F*	

Plan of bed.

The plants weathered the hard winter of 1928–9 and in the spring of 1929 appeared in most cases to be well established. The two rows of plants at the south end of the bed were marked off from the remainder (leaving 25½ ft. of bed), which was then divided up into twelve blocks of eight plants each. The plants in each block were selected at random and marked with the letters *A–H*. The plan of the experiment was to take the flower heads from one plant per block each week, beginning with *A* in the small bud or button stage, and proceeding in this fashion over a period of 8 weeks until finally the *H* plants were taken in the overblown

[1] I am indebted to Mr J. C. F. Fryer, for permission to use this bed and to him and Mr C. T. Gimingham for their constant care and attention to the plants, and for advice during the course of the work.

state. In reality, a rather longer period than 7 days (10 days 19 hours) had to be allowed before the flowers on plants *H* could be regarded as completely overblown. One of the *G* plants (in Block 2) died, and in addition the test on plant *C* in block 4 was spoilt, thus one of the plants had to be wholly and the other partially ignored. The configuration of the bed is given in the plan.

In the plan, the letters were given to assist randomisation and facilitate the proper selection of the plants each week; in the tables, however, in order not to confuse the procedure and to indicate more clearly the week in which the flowers were taken, the plants are given numbers corresponding to the number of the letter in the alphabet. Thus the crop from all the *A* plants taken on May 28th are called the first week's, the *B* plants the second week's and so on down to *G* the seventh week's, while that of the *H* plants, left 1 week 4 days after the collection of the *G* plants, roughly a week and a half, is specified as taken after 8½ weeks.

By the middle of May it was observed that flower buds were forming on the plants; they were, however, too small to give a sufficient weight for analysis. On May 28th the first crop was taken, a certain length of stalk (12–18 in.) being cut, the product of each plant being kept separate, the heads with the stalk were air-dried in the shade in a greenhouse, the temperature of which was not allowed to rise too high and through which a circulation of air was allowed to pass by opening doors and windows. For the first three weeks the heads were all buttons, but on the fourth petals began to emerge. Up to and including the fifth week the heads were separated, after drying, into categories to correspond with their condition at the time of harvesting. This was a comparatively easy matter up to this stage, but afterwards the material was separated immediately after harvesting and the heads in the categories dried separately. After drying, the heads were cut off and stored either in corked tubes or air-tight tins, and the diameter of the receptacle of each head subsequently measured; the receptacles were nearly all practically circular, but in cases where there was a lack of symmetry the mean diameter was determined from two measurements. A piece of cardboard with a V-shaped cut in it and having graduations down the side greatly facilitated measurement in the early stages, but when the flowers were fully open or overblown, callipers were employed. The gross weights of the heads in the different categories were then determined. During the process of measuring it was convenient to detach the last remnant of the stalk, and thus the analyses represented results for flower heads alone. They may have on this account a higher pyrethrin value than those usually found for large samples where it would be almost impracticable to detach the stalk so completely. The advantages, however, of this practice are clear, as there is no confusion likely to arise owing to one sample having a proportionally larger quantity of stalk than another.

The categories. The use of the terms buttons, closed with petals, half-open and overblown heads requires some definition. The difficulties involved in this have already been indicated. In Plate XLIV, however, we have given photographs illustrating the several classes from the button to the fully open stage. Plate XLIV, fig. 1 A, shows them as harvested. Reading from the left we have, first the closed bud or button stage; the two following flowers show the range covered by the term "closed showing petals," the flower 3 being in a more advanced stage than 2; the fourth flower we have regarded as "half open" but in rather an advanced stage, and this category would, in general, include a range of flowers down a stage where the ray florets had just flattened out. The last flower to the right would be regarded by us as just "fully open," the first ring of the disk florets having opened out, but as it was impossible to select flowers in precisely this state, the range would include flowers in which several rings of disk florets were open. Plate XLIV, fig. 1 B, illustrates these flowers after air-drying, and it is obviously not easy by casual inspection to differentiate between the later classes when dry. Plate XLIV, fig. 2, shows the fully open flowers just after harvesting (A) and when air-dried (B) respectively. In the air-dried series (B) it will be noted that the first three flowers to the left have dried with the petals closed over the disk, whereas the three to the right have dried outwards and downwards exposing the disk florets to view. Recording the flower on the extreme left as No. 0, in which no disk florets are open, and proceeding to the right, No. 1 has the first circles from the periphery open, No. 2 the first and second circles, No. 3 all open to the third circle, No. 4 is open to the fourth circle, and in No. 5 all the disk florets are open except a few in the centre. It would appear, therefore, that there is a tendency on drying for the petals to shrivel over and to enclose the head until a certain number of the circles of florets (about half) are open, and when maturity has passed beyond this stage for the petals to shrivel outwards and backwards. It is, however, almost certain that a considerable variation will be found in this regard, and without the examination of a large number of flowers, it would, in the present stage of our knowledge, be unwise to rely upon this characteristic as more than a rough indication of maturity. As a good deal of heterogeneity exists even in a small bed of pyrethrum flowers, it must be assumed that without close inspection flowers ranging over all the above six classes would be taken as fully open.

Plate XLIV, fig. 3, represents flowers in the completely overblown stage; there is no great difference between the appearance of the flowers before and after air-drying.

Meteorological conditions. The weather conditions prevailing during the period of growth of the flowers was remarkably uniform. Little rain fell over many months, and the temperature both of the air and soil was very uniform. The highest temperatures occurred during the last 11 days of the experiment, the maximum averaged over that period being 76° F. and the minimum 52° F.; usually, however, the maximum daily temperature ranged about 62–67° F. and the minimum about 45–50° F. The soil temperatures were slightly lower. The hours of sunshine and the total radiation as Callendar figures, calculated to joules, are given in Table I.

Table I.

Sunshine and radiation figures.

Week	Bright sunshine (hours)	Radiation figures (joules)
1–2	44·8	10,576
2–3	55·8	11,664
3–4	50·1	11,314
4–5	63·4	12,391
5–6	40·8	10,792
6–7	41·1	9,441
7–8½	131·6	22,812

It is possible that the uniformly dry and moderately warm weather prevailing during some critical period of growth had the effect of raising the pyrethrin content of the flowers. The determination of the effect of meteorological conditions, particularly of rainfall, temperature and sunshine, upon flowering and the content of the pyrethrins in the flowers, would appear to be a matter of some importance. It is known that this plant rarely, if ever, flowers in certain countries close to the equator. A direct experiment to determine the meteorological conditions that lead to maximum and minimum flowering and content of the active principles might be of value as indicating the types of climate suitable or unsuitable for the growth of this plant.

The total radiation figures and the sunshine hours for each period are plotted in Text-fig. 1 and the accumulated data, counting from the first period, in Text-fig. 2. There is a considerable rise in the figures during the last week and a half, and this is concordant with a large increase in the weight of the flower heads during this period. It is doubtful whether the correlation is important, as prior to and during this time pollination has taken place and the fruits are forming and a large increase was to be expected. It is, however, a moot point whether it would have been so rapid except for the large amount of sunshine experienced during the period. A correlation between the amount of radiation and the increase in weight and of the pyrethrin content during the periods was not sought, and it would appear as if only a direct experiment in which all the other variations likely to occur in the open are reduced to a minimum could elucidate the part played by sunlight in the development of the flowers and their active principles.

The estimation of the pyrethrin content. The samples were ground to a moderately fine powder and thoroughly mixed for analysis. An attempt was made to analyse the material from individual plants for the third week, but the spoiling of one test and the impossibility of repeating the analysis rendered the pooling of the material necessary. Four individual plants had been analysed in this case, but the remainder of the samples were mixed together, the pyrethrin content determined and the mean values for the pyrethrins calculated for the whole (eleven plants). In view, therefore, of the small amount of material available from individual plants for the first two weeks, flowers for each of these weeks were mixed together, ground and the pyrethrin content determined. In the subsequent weeks the samples from each plant were ground and analysed separately, and in two cases, to be dealt with later, it was possible to do estimations on the flowers in the separate categories. The loss on heating to 103° C. in an electric oven for 24 hours was ascertained, and although it is known that a small amount of volatile oil is present in the fresh flowers, the material after this treatment was regarded as dry matter and the pyrethrin contents per cent. were calculated to this basis. These values are stated in the tables as percentages on the oven-dried heads.

The samples were extracted with petroleum ether (B.P. under 40° C.), and the contents of pyrethrin I and II determined by the micro-method outlined by Tattersfield, Hobson and Gimingham (8). The values, however, came out in the later weeks at so high a level that difficulties were met with in the analyses and some slight modifications of the method had to be introduced. This phase of the problem has been dealt with in a separate paper by Martin and Tattersfield (4). In addition, the copper-reduction method outlined by Gnadinger and Corl (2) and the ferricyanide method of Martin and Tattersfield (*loc. cit.*) were also employed for checking certain values that appeared unusually high. The latter method was devised as a result of the difficulties experienced in dealing with our samples which, in some cases, were much richer than we had hitherto met with; it enabled us to check rapidly the results obtained in the later stages of the growth of the flower heads, but was not so successful for analysing the small buds. The pyrethrin content is expressed in the tables as percentages on both the air-dried and oven-dried heads, and also as the mean amount found per flower head and per plant. There were considerable variations in the values obtained for the flower heads from individual plants in all the weeks where it was found possible to carry out analyses.

In order to test the significance of the results obtained a statistical analysis of the data was carried out by the Statistical Department at Rothamsted. This not only makes it possible to decide how far reliance can be given to the conclusions drawn, but also to put on record in an abbreviated form the large mass of figures accumulated, while giving some indication of the degree of variation existing amongst the detailed data. An analysis of variance, the main procedure employed (1), makes possible through the "*z*" test a determination of the significance of the effect of position of any plant in the bed, and of time of harvesting upon the value being considered and gives a relatively more accurate estimate of the standard errors of single plants or those of the means of twelve plants. Where, however, the data were not suitable for the application of this analysis the standard errors were determined in the usual way. It may be noted here that, when tested, the standard errors of the general mean were not significantly different in value from those determined for the means by plants.

RESULTS.

Numbers of flower heads. The whole of the flower heads on each batch of plants were taken on each occasion. Over the whole of the series there was a considerable amount of variation, the lowest yield on one plant being 18 and the highest 440. The mean numbers per plant based on the crop taken each week are given in Table II.

The analysis was carried out to ascertain whether the mean numbers of flowers varied significantly with the blocks into which the bed was divided or with the dates upon which the flowers were gathered. The "*z*" test indicates that there was just a significant block effect, which varied from 153 to 76 per cent. of the mean yield. There was no effect due to dates, *i.e.* the number of flower heads found are not likely to vary significantly whether taken the first week or the last. It is obvious that the flower buds are laid down in the earlier stages of the annual growth and do not materially increase in number during the course of the experiment.

Table II.

Numbers of flower heads each week.

Week	1	2	3	4	5	6	7	8½
Mean nos. per plant ...	136·2	163·2	155·7	157·7	145·9	162·7	138·7	159·6

Standard error of a single plant = ±63·28; standard error of mean of twelve plants = ±18·27.

An analysis of variance gave the following results:

	Degrees of freedom	Sum of squares	Mean square	½ $\log_e$ mean square
Blocks	11	87,397	7945	1·0362 (z = 0·3427)
Dates	7	9,476	1353·7	
Remainder	75	300,285	4003·8	0·6936
Total	93	397,158		

Degree of maturity of the flower heads. In Table III an attempt is made to give a short representation of the degree to which the maturity of the flowers varied with the date of harvesting. The general mean percentages for twelve plants for the flowers found in each category are given, and alongside them in brackets the range of percentage values among the individual plants. Thus, the flowers taken on June 25th from twelve plants gave a mean percentage of buttons of 8·3, but samples taken from each plant showed a variation ranging between 0·7 and 24·3 per cent. The system of tabulating was adopted since the value of a full table of figures was not commensurate with its complexity.

Table III.

Degree of maturity of the flower heads.

Week (Date of harvesting)	Buttons (mean %)	Closed showing petals (mean %)	Half open (mean %)	Fully open (mean %)	Over-blown (mean %)
1 (May 28)	100	—	—	—	—
2 (June 4)	100	—	—	—	—
3 (June 11)	100	—	—	—	—
4 (June 18)	83·6 (58·6–97·8)	16·4 (2·2–41·4)	—	—	—
5 (June 25)	8·3 (0·7–24·3)	64·7 (30·2–84·9)	20·1 (3·0–52·6)	6·9 (0–25·3)	—
6 (July 2)	2·1 (0–13·1)	16·6 (2·2–49·2)	12·4 (8·4–19·6)	68·9 (28·7–87·6)	—
7 (July 9)	—	2·1 (0–7·7)	6·4 (0–52·8)	91·5 (47·2–100)	—
8½ (July 20)	—	—	—	—	100

The figures represent the general means. The numbers in brackets give the minimum and maximum percentages found.

No attempt has been made to correlate the results expressed in Table III with the pyrethrin contents, but as the average degree of maturity is obviously correlated with the dates at which the flowers were taken, a significant effect on the pyrethrin contents correlated with the dates may well be one due to maturity. There is obviously a good deal of heterogeneity in the flowers taken in the fourth, fifth, sixth and seventh weeks, as judged by their degree of openness. The data for the flowers of the fourth week were tested statistically and a significant variation in maturity among its twelve plants was found. It is hoped that at an early date it will be possible to make a more accurate comparison between the rates of maturation of different plants and to ascertain to what extent genetical factors may be involved.

Size of flowers. It was considered that the diameter of the receptacles of the flowers would give a rough estimate of the size of the flower heads. These data had been accumulated in the way described for each head. The mean diameter of the heads from each plant, the general mean and the mean by plants for each series of twelve plants were calculated. The two latter means are expressed along with their standard errors in Table IV.

Table IV.

Mean diameter of receptacles of flower heads each week.

Week ...	1	2	3	4	5	6	7	8½
General means in mm. ...	4·07	5·59	6·20	7·17	7·86	8·71	9·4	11·7
Means by plants in mm. ...	4·03	5·66	6·21	7·31	7·89	8·81	9·31	11·8
Standard errors	±0·134	±0·135	±0·078	±0·184	±0·146	±0·176	±0·289	±0·264

An analysis of variance for this table was not carried out, but the standard errors for each week's crop were ascertained. The general means were determined by dividing the sum of the diameters by the total number of flowers gathered each week, while the means by plants were ascertained from the weekly sum of the means for each plant, divided by the number of plants from which that week's crop had been taken. There is little difference between these two values and no significant differences between their respective standard errors.

Moisture content of the flower heads. These were determined by drying the powdered flowers overnight in an electric oven heated to 103° C. For the first three weeks the crops from the whole twelve plants were mixed and used for the estimations, but, during the following five and a half weeks the crop of flowers from each plant was tested separately. The mean values are given in Table V.

Table V.

Moisture content of flower heads in percentages.

Week	1	2	3	4	5	6	7	8½
General mean values	11·6	11·2	10·3	10·3	10·7	11·4	11·8	10·6
Mean values by plants	—	—	—	10·65	10·61	11·38	11·78	10·52

Analysis of variance.

	Degrees of freedom	Sum of squares	Mean square	$\frac{1}{2}\log_e$	“z”
Dates	4	15·2326	3·8082	1·81982	0·82698
Blocks	11	7·6498	0·6954	—	—
Remainder	43	31·3194	0·7284	0·99284	—
Total	58	54·2018			

The standard error of a single plant as calculated from the remainder = ±0·85 per cent.
The standard error of the mean of twelve plants = ±0·38 per cent.

The dates upon which the flowers have been taken have had a significant effect, but no effect due to blocks is indicated.

The determination of the moisture content in this way probably gives only an approximate estimation, as the flowers contain small amounts of volatile oil, part of which would be lost at 103° C.; the loss due to this factor is, however, not large.

Weights of flower heads. The average figures for the weights of the heads are set out in Table VI.

Table VI.

Weights of flower heads at each harvesting.

Week of harvesting	Mean weight of air-dried heads per plant (gm.)	Mean weight of oven-dried heads per plant (gm.)	General mean weight of single air-dried head (gm.)	Mean weight by plants of single air-dried head (gm.)	General mean weight of single oven-dried head (gm.)	Mean weight by plants of single oven-dried head (gm.)
1 (May 28)	1·1568 ± 0·18	1·0226 (±0·16)	0·0085	0·0084 ± 0·00066	0·0075	(±0·00058)
2 (June 4)	3·0396 ± 0·28	2·699 (±0·25)	0·0186	0·0195 ± 0·00124	0·0165	(±0·0011)
3 (June 11)	4·759 ± 0·45	4·379 (±0·4)	0·0313	0·0318 ± 0·00204	0·0256	(±0·0018)
4 (June 18)	9·075 ± 0·88	8·140 ± 0·78	0·0575	0·0594 ± 0·0027	0·0516	0·0531 ± 0·0024
5 (June 25)	17·267 ± 2·68	15·416 ± 2·38	0·118	0·115 ± 0·0037	0·106	0·103 ± 0·0032
6 (July 2)	25·531 ± 4·40	22·612 ± 3·87	0·157	0·158 ± 0·0054	0·139	0·140 ± 0·005
7 (July 9)	25·964 ± 3·99	22·890 ± 3·53	0·187	0·180 ± 0·0095	0·165	0·1586 ± 0·0082
8½ (July 20)	41·312 ± 3·80	36·930 ± 3·32	0·259	0·262 ± 0·0092	0·231	0·2345 ± 0·0083

Note. The general mean was obtained by dividing the total weight of flower heads from the twelve plants by the total number, the mean, by plants by dividing the sum of the means for each plant by the number of plants. There was not a significant difference between the standard errors of these means.

In Table VI, the average weight of heads per plant obtained from twelve plants is expressed for both the air-dried and oven-dried (at 103° C.) material. We were not able to ascertain the standard errors for the latter for the first three weeks as the samples had been pooled for purposes of analysis, but it will be observed from the table that although the errors, where determined for the oven-dried heads, are slightly smaller than for the air-dried, there is a close proportionality, from which the values in brackets have been calculated. The remainder of the table gives the average weight of a single head from each week's harvest both for the air- and oven-dried material. The general mean and the mean by plants were obtained in the usual way. In the text-figures we have plotted only the general means of the oven-dried flowers. An inspection of Table VI shows that while, in general, there are rises in the yield of the flowers by weight per plant with the passage of time, the increase in weight between the sixth and seventh week is definitely not significant. It is at this stage that the flowers approach maturity and any considerable increase would not be expected, but the standard errors throughout this portion of the table are rather high, due to considerable variation as regards yield in numbers of flowers per plant and the weights of flowers. An analysis of variance showed that the yield of flowers by weight per plant was just significantly affected by position in the bed, but was profoundly affected by the date. The latter statement conveys the obvious truth that the flower heads will unquestionably increase in weight as they grow to maturity.

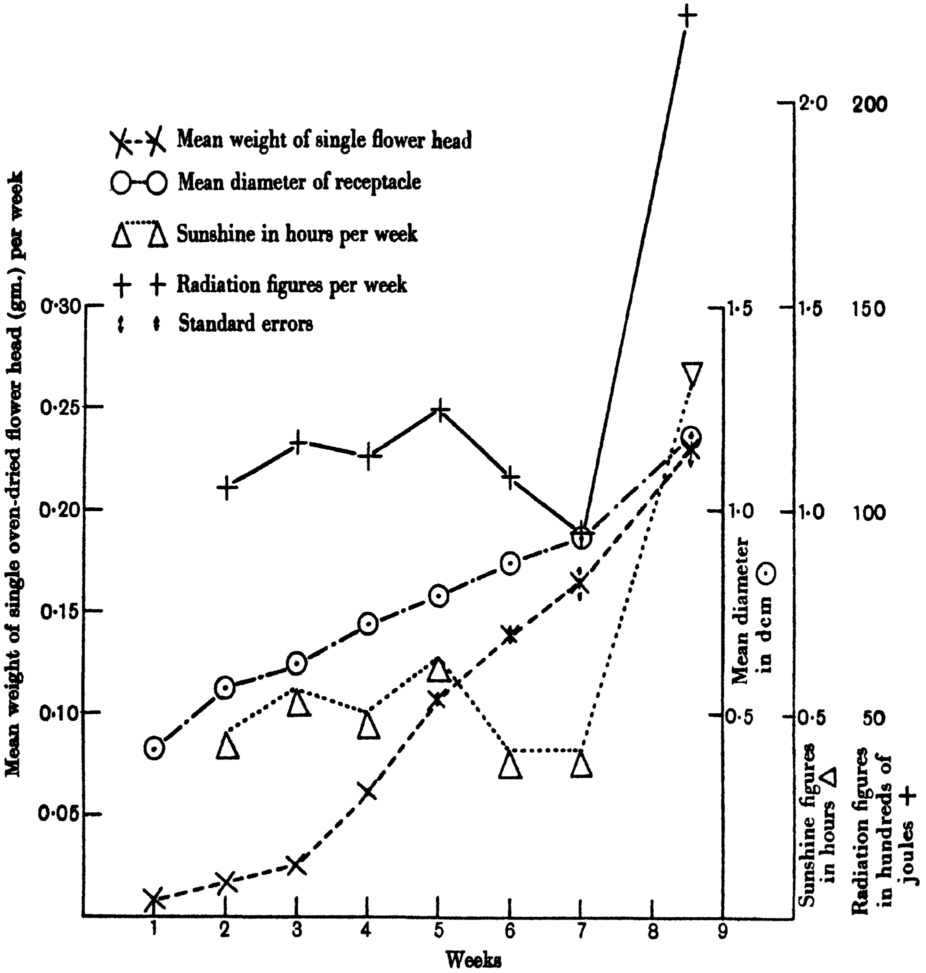
The data in Table VI show that the average weight of a single flower head each week at first increases slowly then more rapidly, and although retardation takes place as maturity of the flowers is approached there is rather a sudden rise after the seventh week, when pollination has been effected. The growth curve is S-shaped, up to the point where pollination has taken place. The weekly average weights of the single flower head are plotted in Text-figs. 1 and 2, together with the radiation figures.

No attempt has been made to seek a correlation between the radiation and growth data. On one season's figures it might well be misleading.

Correlation between mean diameter of receptacles and weight of flower heads. As the weighing of each individual head was impracticable it was considered advisable to ascertain for each category what degree of correlation existed between the mean diameters and the weights of the heads. Dr Wishart kindly determined the correlation coefficients for the whole range, they were:

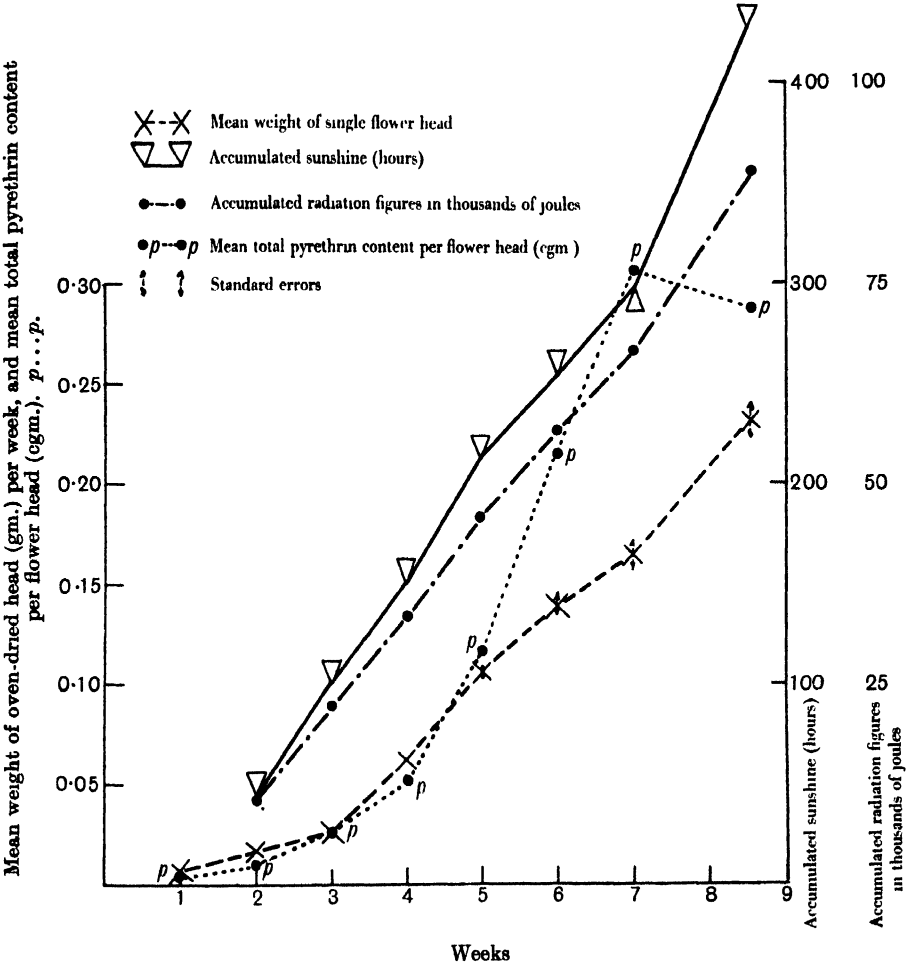
1st week buttons *0·865*. 2nd week buttons *0·885*.
3rd week buttons *0·631*. 4th week buttons *0·621* closed showing petals *0·588*.
5th week buttons *0·861* closed showing petals 0·278 half open 0·319.
6th week closed showing petals *0·690*, half open *0·858*, three-quarters open *0·834*, fully open *0·564* (?).
7th week fully open 0·326 (11 pairs).
8½ weeks overblown *0·872*.

For twelve pairs of values figures greater than 0·576 and for eleven pairs of values greater than 0·602 indicate a significant correlation, they are represented in italics. Thus, there is no doubt that the correlations are significant over the major portion of the period, but begin to fail when the flower opens out. It is probable that the mean weight more truly represents the average sizes of the flowers than the diameter of the receptacle in the later stages of growth, as some difficulty is experienced in measuring the latter values, but that the mean diameter of the receptacle does roughly indicate the range of sizes over the period.



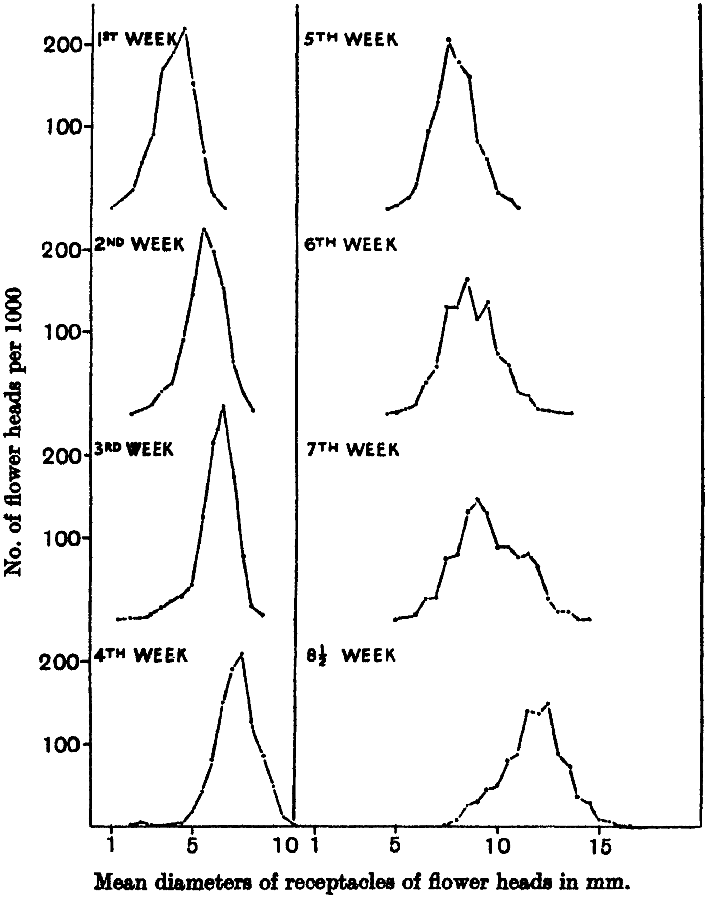
Text-fig. 1. Growth rate of pyrethrum flowers and radiation figures.

The distribution of mean sizes of the flower heads. Text-fig. 3 shows the distribution curves of the size of the flower heads as represented by the mean diameters of their receptacles. Each point represents the numbers of heads per thousand, placed in classes differing in diameter from each other by approximately 0·5 mm. The modes of the curves, as would be expected, move to the right with time, but there is also a tendency for the curves to flatten out after the third week, indicating an increase in heterogeneity with respect to size, which would be expected in the fourth, fifth and sixth weeks, as the buds open out at different rates during these weeks, and there is some overlap in the sizes among



Text-fig. 2. Growth of pyrethrum flowers, and radiation figures (accumulated).

the different categories; it should, however, be noted that in the material in the seventh week there is a further slight increase in heterogeneity of size. In this case the flowers are nearly all open. The distribution curve for the overblown heads taken 8½ weeks after the first crop is significantly to the right of the others, due undoubtedly to pollination and the formation of achenes having given rise to a rapid increase in the size of the receptacles.



Text-fig. 3. Distribution of mean sizes (diameters in mm.) of receptacles of flower heads.

The percentage pyrethrin content. The mean percentage content of the pyrethrins both on the air- and oven-dried heads are given in Table VII.

Table VII.

Percentage of pyrethrin I on air-dried flower heads.

Week	1	2	3	4	5	6	7	8½
General mean	0·12	0·21	0·25	0·435	0·455	0·708	0·75	0·497
Mean by plants	—	—	—	0·441	0·480	0·672	0·700	0·504

Analysis of variance of weeks 4–8½.

	Degrees of freedom	Sum of squares	Mean square	$\frac{1}{2}\log_e$	"z"
Dates	4	0·67246	0·16812	1·4110	0·8740
Blocks	11	0·61884	0·05626	0·8637	0·3267
Remainder	43	1·25872	0·02927	0·5370	—
Total	58	2·55002			

Effect due to date—significant.
Effect due to blocks—not quite significant.
Standard error estimation for single plant ±0·17.
Standard error estimation for twelve plants ±0·049.

Percentage content of pyrethrin I on oven-dried flower heads.

Week	1	2	3	4	5	6	7	8½
General mean	0·135	0·235	0·28	0·485	0·51	0·80	0·85	0·556
Mean by plants	—	—	—	0·493	0·536	0·757	0·795	0·561

Analysis of variance of weeks 4–8½.

	Degrees of freedom	Sum of squares	Mean square	$\frac{1}{2}\log_e$	"z"
Dates	4	0·905108	0·226277	1·55959	0·57474
Blocks	11	0·788551	0·071686	0·98485	0·32700
Remainder	43	1·602772	0·037274	0·65785	—
Total	58	3·296431			

Effect due to date—significant but less than 1 per cent. point.
Effect due to blocks—not quite significant.
Standard error of estimation for single plant ±0·193.
Standard error of estimation for twelve plants ±0·056.

Percentage of pyrethrin II on air-dried flower heads.

Week	1	2	3	4	5	6	7	8½
General mean	0·24	0·38	0·576	0·644	0·525	0·656	0·89	0·643
Mean by plants	—	—	—	0·632	0·566	0·705	0·832	0·635

Analysis of variance of weeks 4–8½.

	Degrees of freedom	Sum of squares	Mean square	$\frac{1}{2}\log_e$	"z"
Dates	4	0·49150	0·12288	1·2542	0·5963
Blocks	11	0·13644	0·01240	—	—
Remainder	43	1·60313	0·03728	0·6579	—
Total	58	2·23107			

Effect due to date—significant but less than 1 per cent. point.
Effect due to blocks—not significant.
Standard error of estimation for single plant ±0·193.
Standard error of estimation for twelve plants ±0·057.

Table VII (*cont.*).

Percentage of pyrethrin II on oven-dried flower heads.

Week	1	2	3	4	5	6	7	8½
General mean	0·27	0·425	0·63	0·718	0·59	0·74	1·00	0·686
Mean by plants	—	—	—	0·707	0·63	0·796	0·944	0·703

Analysis of variance of weeks 4–8½.

	Degrees of freedom	Sum of squares	Mean square	½ $\log_e$	"z"
Dates	4	0·693275	0·173319	1·42627	0·48449
Blocks	11	0·169668	0·015424	0·21666	—
Remainder	43	2·828078	0·065769	0·94178	—
Total	58	3·691021			

Effect due to dates—significant approximately 5 per cent. point.
Effect due to blocks—not significant.
Standard error of estimation for a single plant ±0·256.
Standard error of estimation for twelve plants ±0·074.

Percentage of total pyrethrins on air-dried flower heads.

Week	1	2	3	4	5	6	7	8½
General mean	0·36	0·59	0·824	1·079	0·98	1·364	1·64	1·11
Mean by plants	—	—	—	1·073	1·045	1·376	1·532	1·139

Analysis of variance for weeks 4–8½.

	Degrees of freedom	Sum of squares	Mean square	½ $\log_e$	"z"	Sum of products pyrethrin I and pyrethrin II	"r"
Dates	4	2·15987	0·53997	1·99447	1·03359	0·49795	0·86615
Blocks	11	1·14760	0·10433	1·17247	0·21159	0·19616	0·6751
Remainder	43	2·93805	0·06833	0·96088	—	0·03810	0·02682
Total	58	6·24552					

Effect due to date—significant.
Effect due to blocks—not significant.
Standard error of estimation for a single plant ±0·2614.
Standard error of estimation for twelve plants ±0·075.

The correlation of percentages of pyrethrin I and II for blocks—significant (from "r" test).

The correlation of percentages of pyrethrin I and II for dates—doubtfully significant.

The correlation of percentages of pyrethrin I and II for single plants—not significant.

Percentage of total pyrethrins on oven-dried heads.

Week	1	2	3	4	5	6	7	8½
General mean	0·405	0·66	0·91	1·203	1·09	1·54	1·85	1·24
Mean by plants	—	—	—	1·200	1·168	1·553	1·740	1·265

Table VII (*cont.*).

Analysis of variance for weeks 4–8½.

	Degrees of freedom	Sum of squares	Mean square	½ $\log_e$	"z"	Sum of products pyrethrin I and pyrethrin II	"r"
Dates	4	2·993182	0·748295	1·00631	1·00178	0·697400	0·88040
Blocks	11	1·468745	0·133522	0·14452	0·13999	0·253263	0·69790
Remainder	43	4·542738	0·100950	0·00453	—	0·055944	0·02630
Total	58	9·004665					

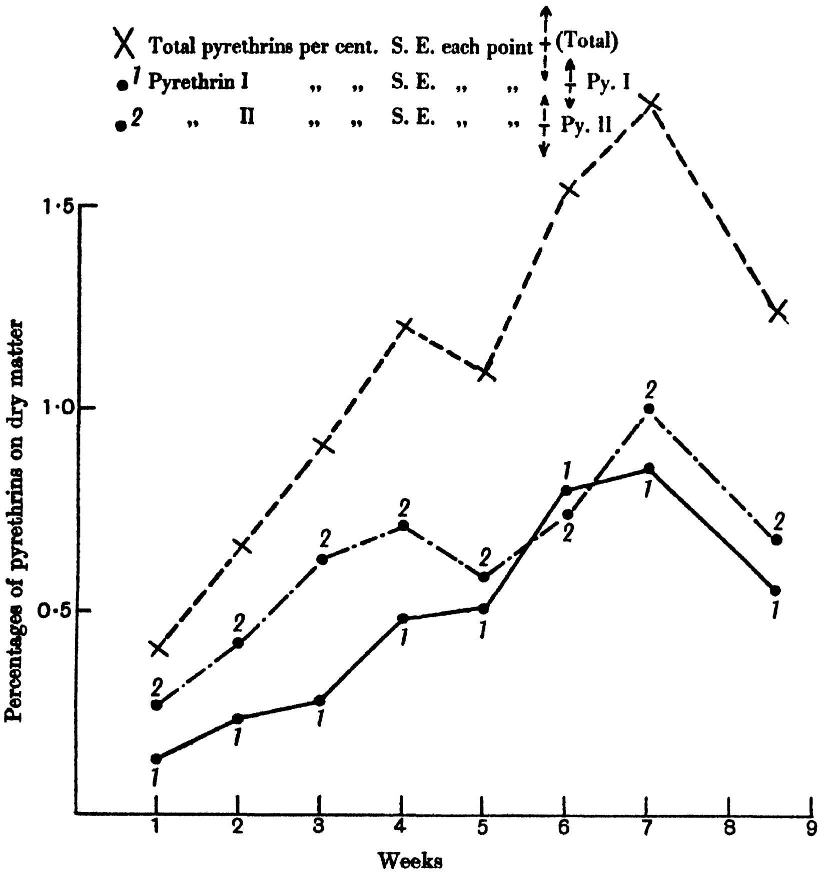
Effect due to date—significant.
Effect due to blocks—not significant.
Standard error of estimation for a single plant ±0·317.
Standard error of estimation for twelve plants ±0·0916.
The correlation of percentage of pyrethrin I and II for blocks—significant (from "r" test).
The correlation of percentages of pyrethrin I and II for dates—just significant.
The correlation of percentages of pyrethrin I and II for single plants—not significant.

The application of the "z" test to the data in Table VII shows that the date upon which the flower heads were gathered had a significant bearing upon their percentage content of pyrethrin I and II, whether considered separately or together, and whether calculated to the air- or oven-dried material. It is true that only the crops taken on the last five occasions have been considered, when individual plants gave a large enough yield for separate analysis, but it is clear that the date effect in the cases of pyrethrin I and II taken separately could occur by chance not more frequently than between 1 in 100 and 1 in 20 times, and less than 1 in 100 times as in the case of the total pyrethrins. The effect is undoubtedly due to maturation. It was possible also that some unevenness in the distribution of the chemical or physical properties of the soil over the plot might have had some effect on the percentage content of the pyrethrins—it was found not to be significant, although significance is approached in the case of pyrethrin I. In addition, in Table VII the degree of correlation was sought for the way in which pyrethrin I and II vary together by means of the "r" test

$$r = \frac{\text{covariance (pyrethrin I . pyrethrin II)}}{\sqrt{\text{variance pyrethrin I} \times \text{variance pyrethrin II}}},$$

the covariance being equal to the sum of the products divided by the number of degrees of freedom. The results of the analysis show that there is a significant correlation for the blocks. For individual plants the correlation was not significant, owing probably to the heterogeneity of the material. The correlation for the dates of harvesting, that is, for maturity, is just about significant, as the values are not likely to occur by chance more frequently than 1 in 20 times.

The general mean percentage figures are plotted against the dates in Text-fig. 4.



Text-fig. 4. Weekly averages pyrethrin content of flowers in percentages, on dry matter.

Table VII and Text-fig. 4 demonstrate that throughout most of the period the flower heads show a higher percentage of pyrethrin II than pyrethrin I, and that the curves follow a more or less parallel course. On the sixth week the mean value of pyrethrin I is higher than that of pyrethrin II—a result largely due to one plant harvested that week having a crop of flower heads with an exceptionally high percentage of pyrethrin I. After maturity, when pollination has taken place, the percentage of the pyrethrins falls.

The pyrethrin content per flower head. The mean contents of the pyrethrins in milligrammes per flower head are given, together with an analysis of variance in Table VIII. Owing to the change in the weight of the flowers with time affecting these values progressively, the standard errors of the means by plants for the last five weeks have been calculated separately instead of the general standard error estimated from the mean square of the remainder.

Table VIII.

Content of pyrethrin I in mg. per flower head.

Week	1	2	3	4	5	6	7	8½
General mean	0·01	0·039	0·077	0·25	0·54	1·11	1·40	1·29
Mean by plants	—	—	—	0·27	0·55	1·04	1·29	1·31
Standard error of mean	—	—	—	±0·037	±0·056	±0·10	±0·14	±0·11

Analysis of variance for weeks 4–8½.

	Degrees of freedom	Sum of squares	Mean square	$\frac{1}{2}\log_e$	"z"
Dates	4	10·423139	2·605785	2·78143	1·68077
Blocks	11	1·775095	0·161372	1·66277	0·56211
Remainder	43	3·889786	0·090460	1·10066	—
Total	58	16·088020			

Effect due to date—highly significant.
Effect due to blocks—significant (about 1 per cent. point).

Content of pyrethrin II in mg. per flower head.

Week	1	2	3	4	5	6	7	8½
General mean	0·02	0·07	0·18	0·37	0·62	1·03	1·66	1·59
Mean by plants	—	—	—	0·37	0·64	1·10	1·53	1·63
Standard error of mean	—	—	—	±0·034	±0·032	±0·072	±0·15	±0·125

Analysis of variance for weeks 4–8½.

	Degrees of freedom	Sum of squares	Mean square	$\frac{1}{2}\log_e$	"z"
Dates	4	14·314665	3·578666	2·94009	1·62472
Blocks	11	0·597962	0·054360	0·84652	—
Remainder	43	5·969552	0·138827	1·31537	—
Total	58	20·882179			

Effect due to date—highly significant.
Effect due to blocks—not significant.

Content of total pyrethrins in mg. per flower head.

Week	1	2	3	4	5	6	7	8½
General mean	0·03	0·11	0·26	0·62	1·16	2·14	3·06	2·88
Mean by plants	—	—	—	0·64	1·19	2·14	2·82	2·94
Standard error of mean	—	—	—	±0·05	±0·08	±0·13	±0·26	±0·17

Table VIII (*cont.*).

Analysis of variance for weeks 4–8½.

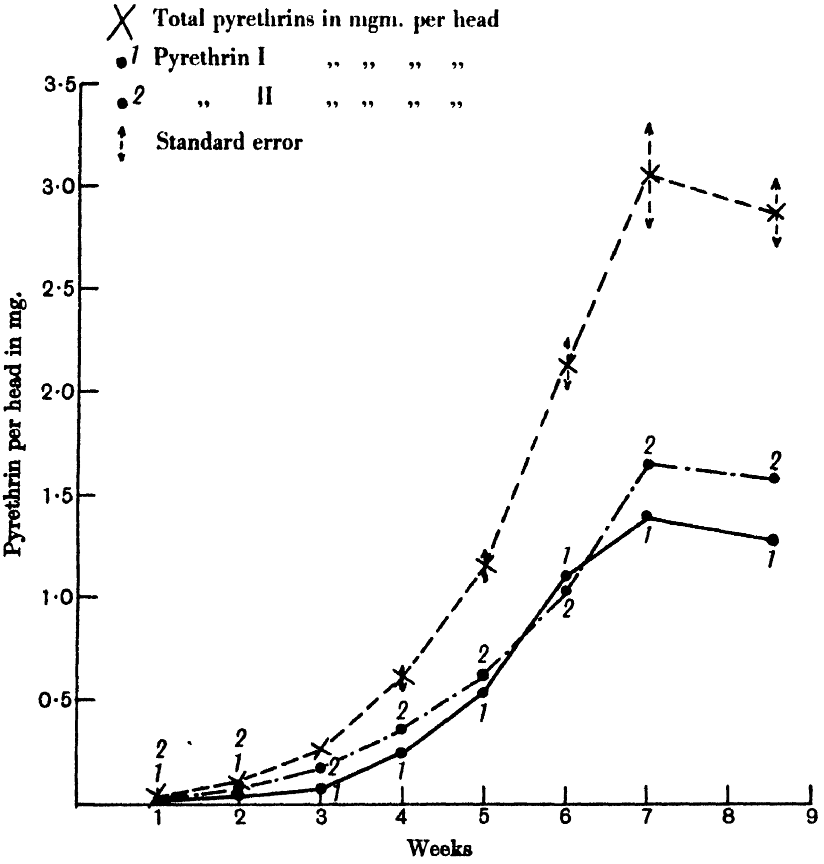
	Degrees of freedom	Sum of squares	Mean square	½ $\log_e$	"z"	Sum of products	"r" correlation
Dates	4	48·897515	12·224379	2·40298	1·88570	12·079855	0·98893
Blocks	11	3·520391	0·320036	0·58157	0·06429	0·573667	0·5568
Remainder	43	12·099865	0·281392	0·51728	—	1·120263	0·2325
Total	58	64·517771					

Effect due to date—highly significant.
Effect due to blocks—almost significant.
Correlation of contents of pyrethrin I and II per flower head for dates—significant.
Correlation of contents pyrethrin I and II per flower head for blocks—significant.
Correlation of contents pyrethrin I and II per flower head for individual plants—not significant.

Table VIII demonstrates the high significance of the date of harvesting upon the absolute yield of the pyrethrins per flower head whether considered separately or in combination, an effect largely due to the degree of maturation. There is also a close correlation with date for the relative amounts of pyrethrin I and II. The effect of the position in the beds (block effect) upon yield of pyrethrin per head only rises to significance in the case of pyrethrin I and has no significance in the case of the total pyrethrins. At this stage it is not possible to offer any explanation for this and too much stress should not be laid upon it. It is, however, important to notice that the degree with which the two active principles vary together is of significance for the various blocks, but not for individual plants, where it is probably neutralised by the general heterogeneity of the material. The data for the pyrethrin content per flower head are plotted against the dates in Text-fig. 5.

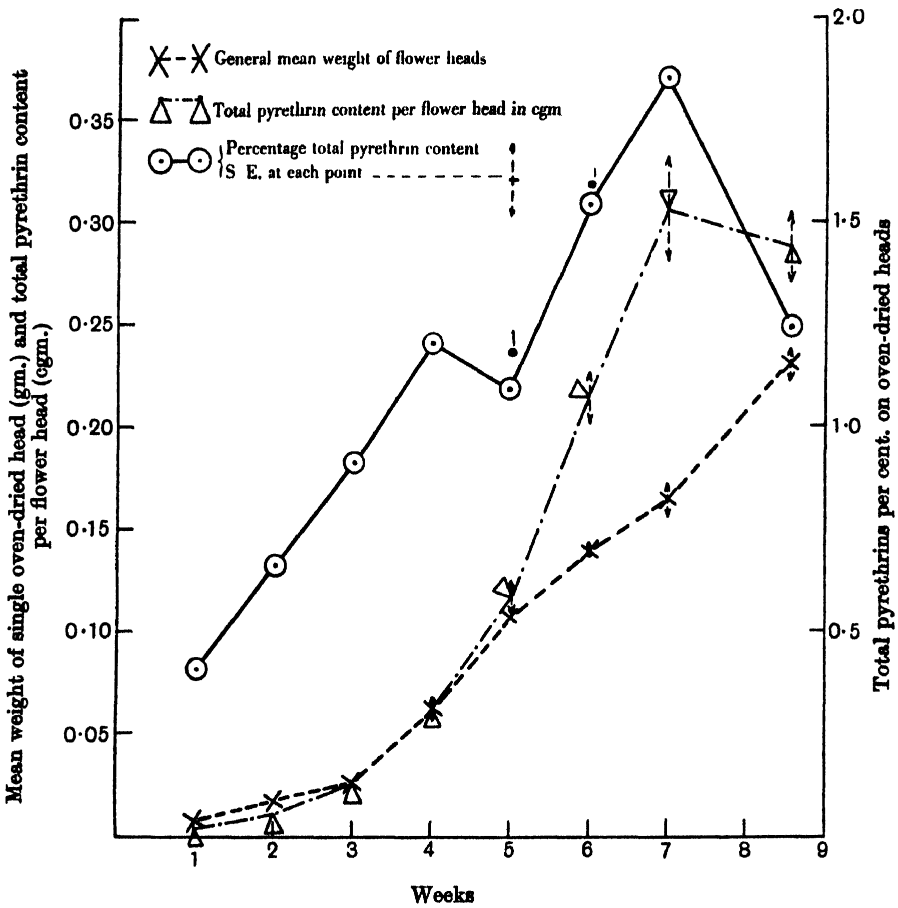
The close similarity of the three curves in Text-fig. 5 is a noticeable feature and brings out the close correlation of the pyrethrin content per flower head with the times of harvesting, shown in the foregoing analyses. The falls in the pyrethrin contents after pollination cannot be regarded as significant, being less than the standard error of the respective means; not only so, but had the means by plants been plotted instead of the general mean a slight but not significant rise in pyrethrin content would have been noted in this region. In Text-fig. 6, the data for the total pyrethrin content per head is plotted on the same diagram as the general mean percentage content of total pyrethrins and also the general average weight of the single flower head taken each week. In addition, there are given figures indicating the average degree of maturity of the flowers taken each week. In all the diagrams, wherever possible without confusion, an attempt is made by means of double arrows to indicate the standard errors of the means.

The points represented by circles indicate the general mean percentage content of total pyrethrins. It may have no significance but the values for weeks 1–4 and 5, 6 are such that a straight line could be drawn to fit them fairly closely. Their most noticeable feature is, however, the fall which takes place between the fourth and sixth week. This is partly due to the presence of an exceptional plant amongst the crop taken on the fifth week; nevertheless, if this value is neglected the general mean only rises to the value represented by the symbol ●′, and the break is still conspicuous. The general mean value for the percentage pyrethrin content for the fifth week is 1·09, the mean of the values for the fourth and sixth week 1·37. The difference between these values which represents the



Text-fig. 5. The pyrethrin content in mg. per flower head each week.

actual fall in pyrethrin content per cent. is 0·26, with a standard error of $0{\cdot}09/\sqrt{2} = \pm 0{\cdot}08$. The decline is therefore significant. Reference to the curve representing the average weight of the single flower head each week shows, however, that the break in the percentage content falls at a period when growth has been most rapid, and it is obvious that the percentage pyrethrin content has not kept pace with the increase in



Weeks	1	2	3	4	5	6	7	8$\frac{1}{2}$
	%	%	%	%	%	%	%	%
State of flowers	100 *B*	100 *B*	100 *B*	83·6 *B* 16·4 *CP*	8·3 *B* 64·7 *CP* 20·1 $\frac{1}{2}$ 6·9 $\frac{3}{4}$–*F*	2·15 *B* 16·55 *CP* 12·4 $\frac{1}{2}$ 68·9 *F*	2·1 *B* 6·4 $\frac{1}{2}$–$\frac{3}{4}$ 91·5 *F*	100 *O*

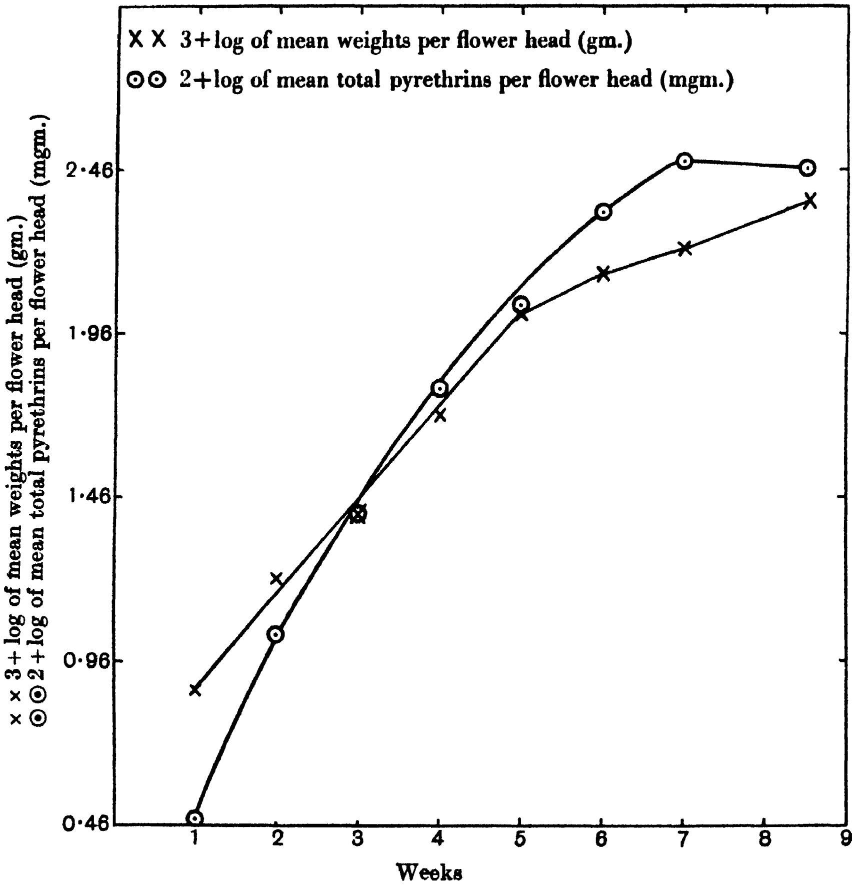
B = buttons, *CP* = closed showing petals, $\frac{1}{2}$ = half open, $\frac{3}{4}$ = three-quarters open, *F* = fully open, *O* = overblown.

Text-fig. 6. Pyrethrin content of flower heads for each week of growth.

weight. The point for the percentage content on the sixth week is also slightly low, but in this case the elimination of one plant, which bore an exceptionally large number of heads slightly less rich in pyrethrin than the average for that week, would bring the general mean value more into line. Although these results may be due to some factor external to the flower, it may be significant that the ray florets contain very little of the active principles, and that during both of these periods the flowers were in the process of opening out, for between the fourth and fifth week, the flowers showing petals have risen from 16·4 per cent. to 91·7 per cent. and between weeks 5 and 6 the flowers, fully open, have risen from 6·9 to 68·9 per cent. The curve representing the general mean content of pyrethrin per flower head shows no break, and from this it is clear that the drop in percentage values does not in any way imply any loss of the pyrethrins in the flower head, but that under our conditions their increase has not been commensurate with the general development of the flower at this stage. Text-fig. 6 demonstrates the fall in the pyrethrin percentage between maturity and the overblown condition. Reference to the growth curve shows that the increase in weight of the flower head at this stage is accentuated by pollination, and since fall in the absolute value of the pyrethrins after pollination is not significant, this increase in weight has apparently not been accompanied by any further synthesis of the active principles, although there has been no actual loss. It is evident, therefore, that the pyrethrin content reaches a maximum value when the flower is fully open, whether considered as percentages or as amounts per flower head.

It is of interest to ascertain the relative rates of increase of weight in the flower heads and of their content of pyrethrins. In Text-fig. 7 we have plotted against the dates the logarithms of the average weight of the single flower head each week and its general mean content of total pyrethrins.

The points showing the relative increase in weight of the flowers (represented in Text-fig. 7 by ×'s) from the earliest stages to near maturity fall close to a straight line drawn between these points. After the fifth week, when the maturation commences, the slope of the curve becomes progressively less steep until pollination occurs between the sixth and seventh week and causes a further accentuation of steepness. The points representing the logarithms of the weekly general means in pyrethrin content per flower head fall on or near a curve the slope of which declines in steepness progressively with time, indicating that in our experiment the relative rate of synthesis of the total pyrethrins has fallen off with time. After the seventh week there is a break in the curve and the value for the overblown flowers after 8½ weeks is not significantly different from that of mature flowers taken at the end of the seventh week. Thus the pyrethrins would appear to accumulate steadily in the flower head with time, although in our case at a progressively retarded relative rate, they more than keep pace with the increase in weight of the head, achieving a maximum content when the flowers are fully open. Although no loss in absolute amount occurs after pollination, the percentage values are lowered by the failure of the plant either to synthesise or translocate the active principles on the flower fading after fertilisation.



Text-fig. 7. Relative increases in weight of head and pyrethrin content.

The pyrethrin content of flowers in different stages of maturity taken at the same time. Two plants harvested in the fifth and six weeks respectively bore a sufficient number of flowers to make possible an analysis of the flowers in varying stages of openness. The data are set out in Table IX.

Table IX.

Analysis of flowers in different stages of maturity taken at the same time.

No. of lant	Time taken, week	Degree of maturity	No. of flower heads	Weight of oven-dried heads (gm.)	Pyrethrin I		Pyrethrin II		Total pyrethrins	
					% on oven-dried heads	Mg. per head	% on oven-dried heads	Mg. per head	% on oven-dried heads	Mg. per head
12	5th	2·1 % buttons 97·9 % closed showing petals	142	12·329	0·25	0·22	0·34	0·30	0·59	0·52
12	5th	97·0 % half open 3·0 % fully open	168	21·745	0·36	0·47	0·42	0·54	0·78	1·01
1	6th	Closed showing petals	89	8·134	0·66	0·60	0·48	0·44	1·14	1·04
1	6th	Half open	68	7·898	0·68	0·79	0·55	0·64	1·23	1·43
1	6th	Three-quarters open	39	4·813	0·73	0·90	0·61	0·75	1·34	1·65
1	6th	Fully open	244	36·002	0·78	1·15	0·60	0·89	1·38	2·04

In the fifth week the heads fell mostly into the categories of "closed showing petals" and "half open" and in the sixth week they ranged from "closed showing petals" to "fully open." Although nothing at present is known of the degree of variation existing between flowers upon the same plant, the analyses indicate that this cannot be very large, but that with increasing maturity there is an increase in the content of pyrethrins. It is true that in our analyses between consecutive categories the increase in pyrethrin content is hardly outside the range of the experimental error, nevertheless the tendency is all in one direction and affords corroborative evidence that the pyrethrin content of the flowers is correlated with the degree of maturity.

Table X.

Contents of pyrethrins in mg. per plant.

Weeks	1	2	3	4	5	6	7.	8½
Mean pyrethrin I in mg.	1·39	6·38	12·1	39·4	78·6	180·9	194	205
Standard error	—	—	—	±5·7	±12·2	±39·6	±35·5	±21·5
Mean pyrethrin II in mg.	2·78	11·5	28·1	58·5	90·6	167	230	254
Standard error	—	—	—	±9·7	±12·0	±18·0	±42·2	±26
Mean total pyrethrins in mg.	4·17	17·9	40·2	97·9	169	348	424	459
Standard error	—	—	—	±11·7	±23·4	±59·8	±75·6	±42

Table X (*cont.*).

Analysis of variance pyrethrin I.

	Degrees of freedom	Sum of squares	Mean square	$\frac{1}{2}\log_e$	"z"
Dates	4	272,854·08	68,213·52	2·11132	1·12843
Blocks	11	123,877·48	11,261·59	1·21062	0·22773
Remainder	43	307,041·79	7,140·51	0·98289	—
Total	58	703,773·35			

Analysis of variance pyrethrin II.

	Degrees of freedom	Sum of squares	Mean square	$\frac{1}{2}\log_e$	"z"
Dates	4	349,920·61	86,980·15	2·23284	1·32617
Blocks	11	123,885·38	11,262·31	1·21067	0·30400
Remainder	43	263,629·20	6,130·91	0·90667	—
Total	58	734,835·19			

In both cases the effect due to dates is clearly significant, but there is no block effect.

Analysis of variance total pyrethrins.

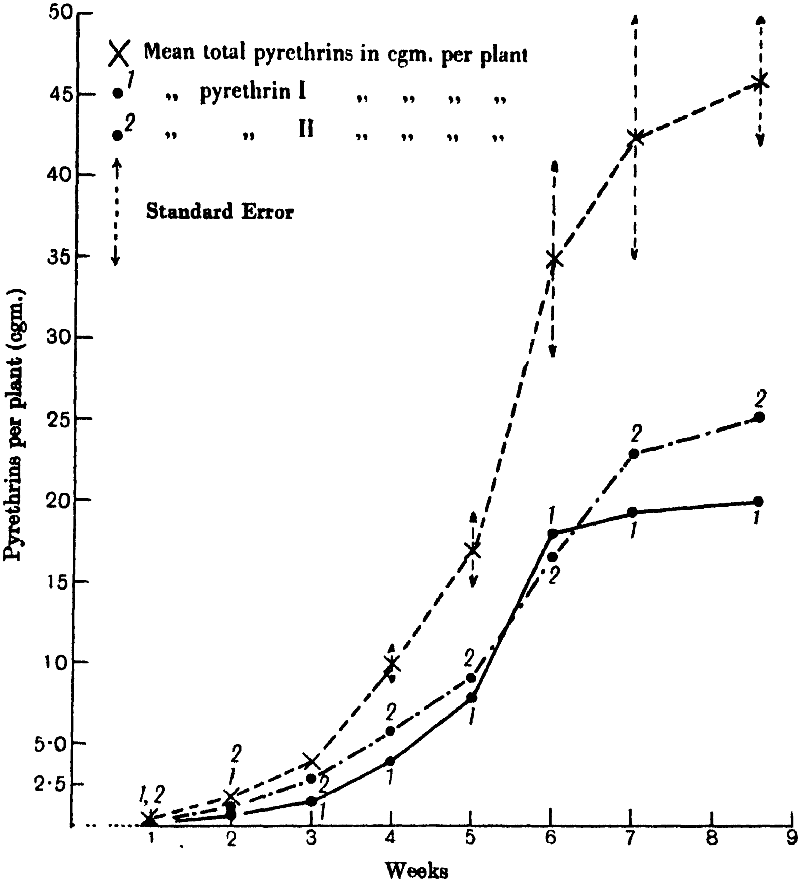
	Degrees of freedom	Sum of squares	Mean square	$\frac{1}{2}\log_e$	"z"	Sum of products	"r" (correlation)
Dates	4	1,214,005·90	303,501·48	1·70640	1·28251	296,615·6	0·96269
Blocks	11	470,093·83	42,735·80	0·72622	0·30233	111,165·5	0·89826
Remainder	43	1,003,818·50	23,344·62	0·42389	—	216,573·8	0·76121
Total	58	2,687,918·23					

Effect due to date—clearly significant.
Effect due to blocks—not significant.
Correlation of contents of pyrethrins I and II per plant for dates—significant.
Correlation of contents of pyrethrins I and II per plant for blocks—significant.
Correlation of contents of pyrethrins I and II per plant for individual plants—significant.

The pyrethrin content per plant. From the point of view of the grower of pyrethrum the efficiency of the plants in the production of the pyrethrins is of importance. In Table X are set out the data and their analysis for the mean yield of pyrethrins per plant for the 12 plants from which the flowers were taken each week, together with the standard errors of the means for weeks 4–8½.

The results are plotted in Text-figs. 8 and 9.

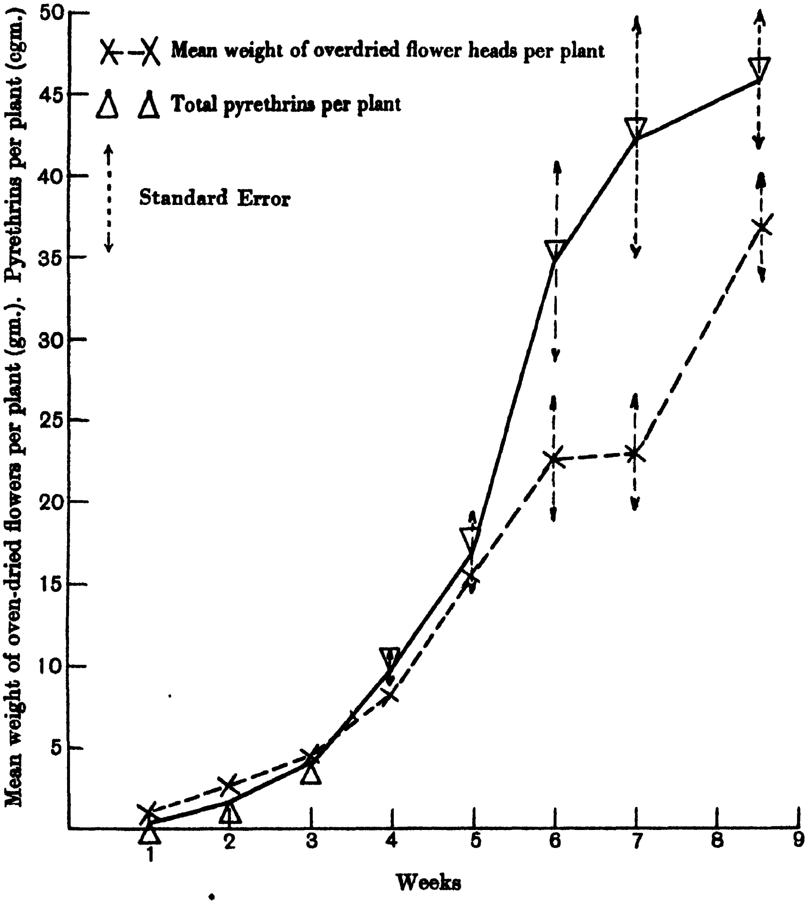
It should be noted that the rises in the pyrethrin contents between the seventh and 8½ weeks are not significant as they are less than the standard error of the mean. The apparent increase in the pyrethrin content per plant after pollination and the withering of the flowers is thus largely due to chance or the heterogeneity of the material. In Text-fig. 9 the weight-growth curve takes the usual S-shape and the increases are significant up to the sixth week, where a marked flattening takes place; the mean weights per plant for the seventh week are not significantly different from those of the sixth, subsequently a large rise in weight takes place due to the fertilisation of the flowers and the formation of seed.



Text-fig. 8. Average pyrethrin content in cgm. per plant each week.

DISCUSSION AND CONCLUSIONS.

The toxicity to insects of pyrethrum flowers in various stages of growth has been examined by several investigators, and the view that "half-open" flowers are more potent than the "open" has been shown to be very doubtful. An examination of the problem, from the point of view of the pyrethrin content by Gnadinger and Corl (3), has demonstrated for *Pyrethrum roseum* a rise in the pyrethrin content (dry basis) of the combined active principles from the small unopened bud stage to the fully opened flower and for *C. cinerariaefolium* that the so-called "closed" flowers have a lower pyrethrin content than the "open" flowers[1]. These conclusions are generally confirmed by our work. There has, however, been in the past some confusion in the terminology "fully open," "half open," "closed," owing to these descriptions having been applied to dry commercial samples. A number of growers have been led to take their crops at a comparatively early stage with the consequent loss of yield and pyrethrin content. These terms have a doubtful validity for the growing flower, *e.g.* Southall's *Materia Medica*



Text-fig. 9. Yield of flower heads, and total pyrethrin per plant.

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[1] Fryer, Tattersfield and Gimingham (*Ann. App. Biol.* 1928, xv, 423) had previously found by biological experiments that, weight for weight, the toxicity of "half open" was no greater than that of "fully open" flowers; and concluded that the correct time to harvest the flowers in practice is at the stage when the majority are "fully open."

describes the "closed" flowers as fully developed. In an article on "The pyrethrum industry in Japan" (5), the British Vice-Consul at Seoul states that the condition for picking is when the flower heads are 70 per cent. open. It is not clear whether this phrase describes the actual condition of the flower or of the crop as a whole. The writer, however, emphasises that loss of yield both of crop and active principles follows the practice of taking the harvest before the flower heads have partially opened. This is unquestionably true, but so long as the confusion of terms lasts, economic loss is likely to follow. We have attempted in our plates to give some visual actuality to our ascriptions, which are based on the flower before drying. There seems little doubt that the yield of pyrethrins as well as of flowers is at a maximum when they are in the fully opened state in the field. There is, however, a period during which the disk florets of the open inflorescence are themselves progressively opening, and it would appear that the extent to which this has happened determines the appearance of the flower when dry. No attempt has been made here to give the pyrethrin content for the flower heads in these different degrees of fully "openness." In some work carried out subsequently and published with J. T. Martin (*loc. cit.*), an attempt has been made to differentiate between them, and it appeared that with our material, up to the stage when all the disk florets were mature, there was a rise in pyrethrin content. From our plates, the flowers with more than one-half the total number of disk florets mature would in the dry state appear to warrant the description of being in the fully opened stage, in that the ray florets dry outwards and backwards, whereas those flowers in which the disk florets are only open to an extent of approximately half this number would apparently be regarded as "half open" when dry. Despite the slight superiority of the former it would be impossible in practice to select a large crop just at the optimum stage, and other countervailing factors might render it advisable despite some loss of weight to take the flowers somewhat earlier—thus the controversy over the respective values of the two categories might well become very artificial. It is evident, however, that for the grower a diminished yield both of flowers and active principles would result from taking flowers before the ray florets were well expanded.

Our plot, although of restricted size, showed considerable heterogeneity among the plants both in yield and in the pyrethrin content of the flowers. On certain plants flowers were borne which had in an early stage of development a higher pyrethrin content per cent. than others at a later stage of development. It is clear, since a wide heterogeneity in this respect is likely to occur in any single crop and to be accentuated when comparisons are made between different crops grown under varying conditions as to climate and cultivation, that selection other than by direct analysis for the pyrethrin content or by biological test is to be deprecated.

In our view no useful purpose is served by taking the flowers to the overblown stage; in our material the increase in yield was largely neutralised by the fall in the percentage content of the pyrethrins, although its absolute amount per flower head suffered little change. Whether this were due to some partial loss of the achenes in harvesting it is not possible to say, but it appears improbable; since, however, in the overblown state the achenes[1] can readily be lost, the inadvisability of prolonging growth to this stage is obvious.

An attempt has been made by a statistical analysis of the data to show whether certain of the correlations examined were significant. The heterogeneity of the material rendered this imperative. The standard errors of the mean were where possible determined from the analysis of variance; this gives a more accurate estimation applicable to the whole table examined. Where, however, the errors progressively changed with time they were determined for the weekly values when data were available. In the main, however, the effects of two factors have been examined, that due to date and that due to the position of the plant in the bed. Although on examination it was ascertained that there was a significant variation in the maturity of flowers taken from different plants on the same date, on the average, the date of harvesting is a measure of the degree of maturation. The position of a plant in a bed may be one of considerable importance, for rarely, if ever, do the factors, soil or meteorological, have an equal effect on plant growth over the whole of even a small bed. The policy adopted of randomising the plants over blocks, the sum of which make up the plot, enables one to compute the significance of the effect due to the position. An analysis has shown that the effect due to date has been significant in the following cases—the weight yield of the flower heads and their mean weight, the moisture content, the percentage content of pyrethrin I, pyrethrin II and total pyrethrins both on air- and oven-dried heads, the content per flower head and per plant of the pyrethrins, both separately and together, and *not* significant in the case of the mean number of flower heads per plant. The effect due to position (block effect) was significant in the cases of the mean number of flower heads per plant, and the content per flower head of pyrethrin I and nearly so for the total pyrethrins per head; there was no significant effect in other cases.

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[1] Gnadinger and Corl have shown the achenes to contain 90 per cent. of the active principles (3).

The way in which the two pyrethrins vary together was also examined, the correlation for dates between the contents of pyrethrin I and II was significant, whether expressed in percentages, parts per flower head or parts per plant. When expressed in percentages, the correlation of the content of pyrethrin I and II for the blocks was significant, but not for individual plants, when expressed in parts per flower head the correlation was nearly significant for blocks but not for individual plants. The material was too variable in character to show an individual correlation for separate plants. When, however, the values were expressed in parts per plant a significant correlation was found for both blocks and individuals.

The data accumulated enable one to conclude for the material examined that there is a quantitative development of the active principles in the flower heads of pyrethrum from the small bud stage up to the time of maturity of the flowers. The synthesis of the active principles more than keeps pace with the increase in weight of the heads, and although fluctuations may take place at periods when rate of synthesis is not commensurate for a time with the increase in weight, the content of the pyrethrins both relatively and absolutely rises to a maximum at the maturity of the flowers. In our experiment the mean percentage content fell after fertilisation and the development of seed, but the amount per flower head remained constant.

Despite the rather high heterogeneity of our material, the average weight of the flower heads up to maturity followed an S-shaped curve usually associated with growth rates; after pollination, however, a rapid increase in weight was observed as seed developed. The curve for the mean content of pyrethrin in parts per head was also of a sigmoid type though less clearly defined. The relative mean weekly increases in weight of flower head were approximately linear in their course for several weeks—but as the flowers approached maturity the slope of the curve declined until fertilisation caused a further accentuation in steepness. While, however, the weight added to the flower head each week was roughly proportional to the weight already accumulated, the pyrethrin content in parts per head follows a course which indicated the relative rate of increase to be slowly declining. This may be due to lack of homogeneity in the flowers, but it is noteworthy that no abrupt break appears in the logarithmic curve until after maturity, at which point further synthesis of the active principles ceased altogether.

It may perhaps be worth notice that two varieties of flowers were observed on the bed, one having the usual appearance, and the other extremely small petals. The factor making for short petal was a genetical one, but so far has not been observed to be linked with high or low pyrethrin content.

SUMMARY.

1. An account is given of the examination of the flowers of pyrethrum plants (*C. cinerariaefolium*) grown upon a bed in Harpenden. The plants were divided into blocks and randomised, the flowers being harvested from a dozen plants each week over a period of 8½ weeks, the flower heads ranged from the small bud stage in the first week to the overblown stage in the last week. The categories into which the flowers were divided to indicate maturity are defined and illustrated.

2. The yield in numbers and weight of heads per plant, the diameters of the receptacles and the content of pyrethrin I and II were determined. There was a considerable amount of variation in all these factors in the flowers from different plants.

3. A statistical analysis showed in this experiment:

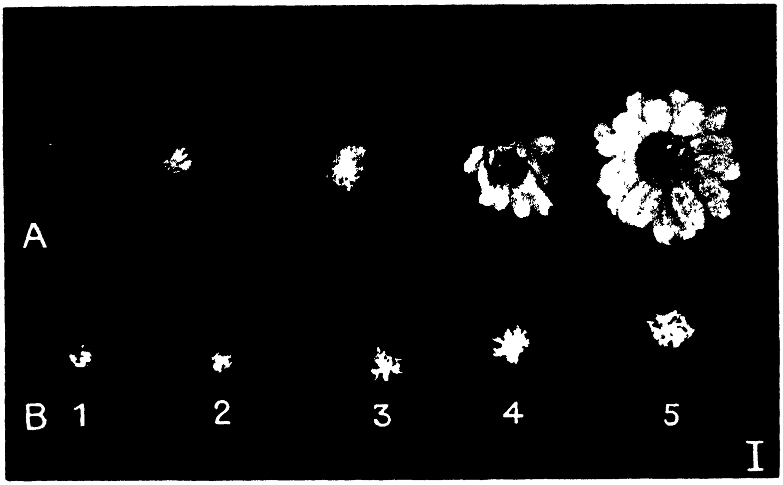
(*a*) That there was no significant variation in the numbers of the flowers with time, but that position of the plant in the bed had a significant effect.

(*b*) That the time of harvesting had a significant effect upon the content of the pyrethrins whether taken separately or together and whether expressed in percentages, parts per flower head or parts per plant. The effect due to position was only definitely significant in the case of pyrethrin I when expressed in parts per flower head.

(*c*) That there was on the different dates a significant correlation between the contents of pyrethrin I and II expressed in parts per flower head or plant.

(*d*) That there was, for the blocks of material examined, a significant correlation between the contents of pyrethrin I and II, whether expressed in percentages, parts per head or parts per plant, but that owing to the heterogeneity of the material the correlation was only significant for individual plants when the pyrethrins were expressed in parts per plant.

4. The data in this experiment indicate that for the material examined, there is a quantitative development of the active principles in the flower heads from the small bud stage up to the time of maturity of the flowers, which more than keeps pace on the whole with the increase in weight of the flowers. Thus the content of pyrethrins, both relatively



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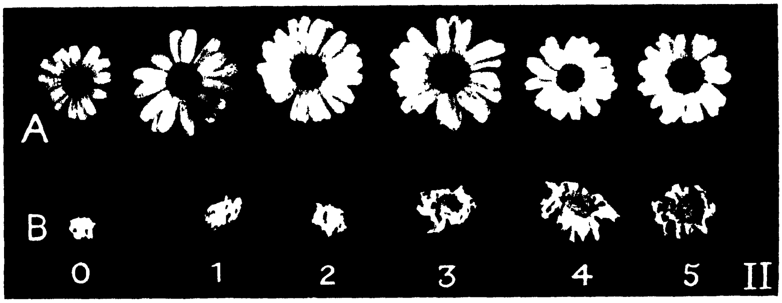
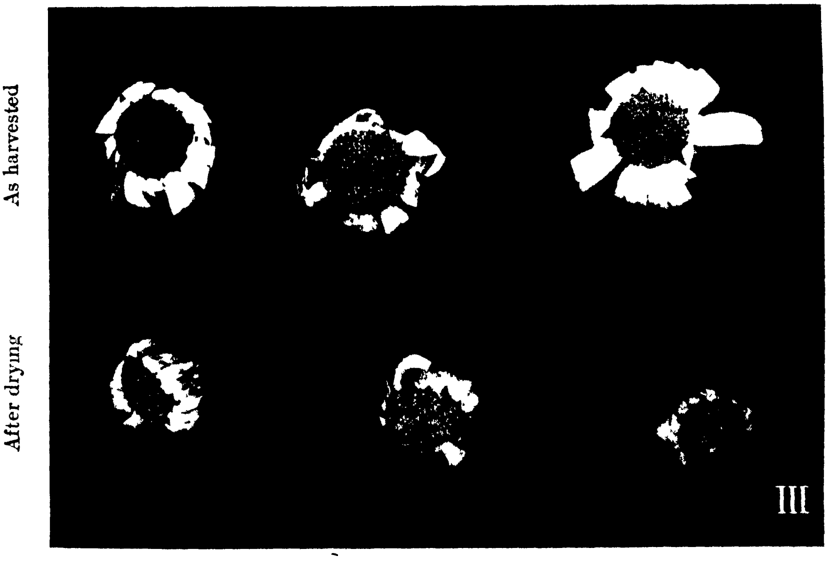


II



III

and absolutely, rises to a maximum at the maturity of the flowers. The mean percentage content of pyrethrins fell after pollination and the fading of the flowers; this corresponds with the rapid increase in weight of the heads on the formation of seed. There would thus appear to be a loss, which might be serious, both in percentage content of active principles and in yield of flowers if harvested before being fully open. There seems to be no useful purpose served in leaving the flowers to the overblown condition.

I wish to express my great indebtedness to Dr R. A. Fisher and Dr J. Wishart for carrying out the greater part of the statistical analyses incorporated in this paper and for much helpful advice. I am also indebted to Mr J. T. Martin for checking many of the analyses by an alternative method.

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EXPLANATION OF PLATE XLIV.

Fig. 1 shows the categories into which the flower heads were divided for purposes of classification. Series A shows appearance just after harvesting, B after air-drying; No. 1 shows the closed bud or button stage; Nos. 2 and 3 closed bud showing petals. No. 4 half-open flowers; No. 5 fully open flowers.

Fig. 2 shows a range of fully open flowers. Series A gives appearance just after harvesting. Series B after air-drying. In flower No. 0 no disk florets are open; No. 1 has the first circle of disk florets open; No. 2 the first and second circles are open; No. 3 all are open to the third circle; No. 4 all are open to the fourth circle; No. 5 all are open except a few florets in the centre. When about half the number of circles of disk florets have opened the ray florets have dried outwards and backwards.

Fig. 3 represents flowers in the completely overblown stage both before and after drying.

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A COMPARISON OF THE EFFECT OF RAINFALL ON SPRING- AND AUTUMN-DRESSED WHEAT AT ROTHAMSTED EXPERIMENTAL STATION, HARPENDEN.

BY "ALUMNUS."

(With Five Text-figures.)

INTRODUCTION.

IN connection with the interpretation of the regression curves of wheat yield on rainfall at different times of the year, obtained by Fisher from the records of the Broadbalk wheat field at Rothamsted (1), a noticeable feature is the large loss of crop ascribable to winter rain, even on plots receiving a substantial spring dressing of nitrogenous manures. It has recently been suggested that this feature might be ascribed to a circumstance not noted in previous papers on the subject, namely that for the 24 years 1854–77, the whole of the ammonium sulphate was applied in the autumn. For six succeeding years the application was made wholly in the spring, and it was not until 1884 that the current system of applications was adopted, in which ½ cwt. per acre is applied in the autumn, and the remainder, which may be ½, 1½ or 2½ cwt., in the spring.

If the large effect observed in the sequence 1854–1918 as a whole were ascribable to the special conditions of the first 24 years, partially counterbalanced as these were by the following 6 years, it is obvious that the effect of winter rainfall in the early period would have to be very pronounced. It was thought worth while, therefore, although the indications from the early 24 years must be subject to large sampling errors, to recalculate the response to rain independently for the years 1854–77. The present paper deals with the following points:

(*a*) The method employed, which is essentially that developed by Fisher, adapted to the shorter run of years now treated.

(*b*) The regression curves obtained for the first 24 years, and the non-significance of the seasonal variation of all but one of the response curves for this short period.

(*c*) Comparison of the average effects of rain over the whole year, with those obtained for the whole period up to 1918. Constant differences will be shown to exist, closely related to Fisher's classification of the plots according to relative abundance of nitrogen, but unrelated to the quantity of nitrogenous manure transferred from the autumn to the spring dressing.

(*d*) Such indications as the response curves give suggest that the additional loss caused by rain in the early period of the history of the field occurred more in the summer than in the winter.

Following the investigation into the effect of rainfall for this period, a further study was made for the period 1879–1930. In 1878 when the change was made from autumn to spring dressings, the authorities very wisely decided to continue the old treatment on plot 15. This plot has since received a dressing of nitrogenous manure at the rate of 2 cwt. per acre each year in the autumn, and thus provides data for a comparison of spring and autumn dressings. Plots 7, 13 and 17 + 18 (ammonium series) are suitable for comparison with plot 15, as they each receive 2 cwt. of nitrogenous manures each year. In this investigation regression curves were found showing the influence of rainfall on the difference in yield between plots 7 and 15, plots 13 and 15 and plots 17 + 18 A and 15. The salient features to be noticed are:

(1) The marked similarity in the regression curves for the differences in yield.

(2) The fact that only one of these curves shows significant seasonal variation, but that this seasonal variation is also significant in the curve for the average difference in yield.

(3) Direct confirmation of the result described above in (*d*), in so far as additional rain in the early summer months is less detrimental to plots which receive spring dressings of nitrogenous manures than to those which have their nitrogen applied in the autumn, while winter rainfall has little effect on the difference in yield.

SUMMARY OF METHOD.

The rainfall for the period is defined by six distribution values for each year a', b', c', d', e' and f', where a' represents the average amount of rainfall falling in each of the sixty-one 6-day periods into which the harvest year is divided; b' represents the linear component of a polynomial fitted to the actual rainfall values for the year; c' represents the quadratic component, and so on.

With these six values, regarded as six independent variates, the yield is to be correlated, but it is first necessary to eliminate the secular trend of each variable; this is done by fitting polynomials to each variable and then correlating the residuals. The values of a', b', etc., were taken from Table IV (1).

A cubic was fitted to each variate; it is not necessary to calculate the polynomial values or the residuals. The sums of squares and products of residuals of the independent variates are now needed, and are found by taking the crude sums of squares and products and applying the appropriate corrections (2). These corrected sums of squares and products were tabulated and correspond to Table XI (1).

Table I shows these values for the period 1854–77 divided by 10^4.

Table I. *Corrected sums of squares and products of deviations*, $\div 10^4$.

	a′	*b′*	*c′*	*d′*	*e′*	*f′*
a′	10·717478	1·131322	−0·062866	0·474546	−1·009653	0·033484
b′	1·131322	3·255947	0·155786	−0·411529	−0·102455	−0·630288
c′	−0·062866	0·155786	1·045280	−0·479302	0·182609	−0·104345
d′	0·474546	−0·411529	−0·479302	1·411041	−0·137535	0·273635
e′	−1·009653	−0·102455	0·182609	−0·137535	0·878959	−0·187534
f′	0·033484	−0·630288	−0·104345	0·273635	−0·187534	0·735976

As we require to find the regression on each of these variates of the wheat yield from several plots, it is necessary to invert the determinant above so as to obtain a matrix of multipliers, each of which is the cofactor of the number above divided by the value of the determinant. This can be done by solving six sets of six simultaneous equations (2), or by building up the adjugate determinant and by dividing each number by the value of the original determinant (3). There is little to choose between the two methods, but the average worker will find the first method the shorter. The results are shown in Table II.

Table II. *Co-factors for determining partial regressions.*

	a′	*b′*	*c′*	*d′*	*e′*	*f′*
a′	+0·110843	−0·037783	−0·030564	−0·048128	+0·123359	+0·007594
b′	−0·037783	+0·397543	−0·012204	+0·067592	+0·088116	+0·337766
c′	−0·030564	−0·012204	+1·171411	+0·391408	−0·228630	−0·046763
d′	−0·048128	+0·067592	+0·391408	+0·919771	−0·035010	−0·235323
e′	+0·123359	+0·088116	−0·228630	−0·035010	+1·419649	+0·412193
f′	+0·007594	+0·337766	−0·046763	−0·235323	+0·412193	+1·833550

For each plot the sums of products of yield residuals and residuals of *a′*...*f′* were calculated as shown above. The values obtained for these sums of products for plot 2B are

a′	*b′*	*c′*	*d′*	*e′*	*f′*
−4369·78	−564·53	+139·49	+241·14	+508·83	−25·69

These values, multiplied by the values of any column of Table II and added, give the regression of yield on the corresponding rain variate divided by 10^4, which was taken out before tabulating the values of Table I. For plot 2B the corrected regressions are

a'	b'	c'	d'	e'	f'
−0·0393114	−0·0041165	+0·0094331	−0·0006814	+0·0099526	−0·0011088

rainfall being measured in thousandths of an inch.

Now if a' had been the only measure of rainfall, the regression of yield on a' would be given by

$$\frac{-4369{\cdot}78}{107174{\cdot}78} = -0{\cdot}0407725,$$

a value not very different from that obtained above; this shows that the estimated average effect of rainfall at all times of the year is little changed by taking the distribution into account. It is important to test whether the distribution terms beyond a' have added appreciably to the value of the prediction of the yield.

ANALYSIS OF RESULTS.

The six regressions can be tested for significance (2) in the following manner, the data being taken from plot 2B.

Variance due to	Degrees of freedom	Sum of squares	Mean square	$\frac{1}{2}\log_e$
Regression on a' only	1	178·1667	178·1667	3·74264
Increase on introducing $b' \ldots f'$	5	2·5119	0·5024	0·80711
Regression on $a' \ldots f'$	6	180·6786	—	—
Deviations from regression function	14	210·5465	15·0390	2·50649
Total	20	391·2251	—	—

The sum of squares due to regression on a' only is found from

$$\frac{(4369{\cdot}78)^2}{107174{\cdot}78}.$$

The sum of squares due to the regression on all six variates, is given by the sum of the products due to the regression function, *i.e.*

$$(-4369{\cdot}78 \times -0{\cdot}0393114) + (-564{\cdot}53 \times -0{\cdot}0041165) + \ldots.$$

The total sum of squares is the sum of squares of yield residuals.

Applying the z test to the total effect of rainfall

$$z = 3{\cdot}74264 - 2{\cdot}50649 = 1{\cdot}23615,$$

while chance will allow z to exceed 1·0909 once in 100 trials, so that the regression on a' gives clearly significant information about rainfall, namely that the yield is reduced by

$$\frac{0\cdot0407725 \times 1000}{61} = 0\cdot668 \text{ bushels per acre}$$

for each additional inch of rain above the average.

The test for the five remaining components of rainfall distribution clearly shows no significant effect of these components; indeed the z test on this plot makes the effect of these components significantly subnormal, *i.e.* less than should appear by chance in five components unrelated to the final yield. No other plot shows this subnormality, and in all plots except plot 6 the differential effect of rainfall at different times of the year cannot be detected in the record of the 24 years examined.

The test for the significance of the regression on a' was applied to each plot in turn with the same conclusion in all cases as to the significance of loss occasioned by additional rain. The data thus allow us to compare the mean decrease in bushels per acre for each plot for the first 24 years with that for the total period, using in each case the average effect of rain throughout the year. In the majority of cases it was found that there was a greater decrease for each additional inch of rain during the early history of the field.

COMPARISON WITH PREVIOUS RESULTS.

Since most or all of the plots during the early period were undoubtedly in a different state of fertility in respect of soil nutrients, and possibly also owing to change in cultural practice, it cannot definitely be inferred that the greater average loss occasioned by rain was wholly due to the change in time of application of sulphate of ammonia, which was the immediate aim of this enquiry. Some light should, however, be obtained by comparing the difference in average loss ascribable to rain between the two periods in the different plots. These plots may be classified either (*a*) by the amount of sulphate of ammonia transferred from the autumn to the spring dressing, or (*b*) according to the rain response curves found by Fisher which conform closely to the degree of nitrogen abundance in the different plots. Grouping the plots as in (*b*) it is noticeable that the greatest difference occurs in plot 6 which has the least nitrogenous dressing, and that this difference decreases with increased nitrogen as can be seen from Table III. The first three plots shown are those in which no change takes place in the application of manures between the two periods.

Table III.

Plot	Treatment	Mean decrease in yield for each additional inch of rain, 1st 24 years	Mean decrease in yield for each additional inch of rain, total period	Difference		Amount of nitrogenous dressing transferred from autumn to spring
		bushels per acre	bushels per acre			cwt.
17 + 18M	No nitrogenous dressing	0·401	0·394	0·007		Nil
5	No nitrogenous dressing	0·278	0·341	−0·063		Nil
2B	Dunged plot	0·644	0·672	−0·028		Nil
10	Double N	0·413	0·421	−0·008		1½
11	Double N + superphosphate	0·717	0·499	0·218		1½
8	Treble N + minerals	0·896	0·719	0·177	Av. 0·251	2½
12	Double N + superphosphate + sulphate of soda	1·150	0·917	0·233		1½
14	Double N + superphosphate + sulphate of magnesia	1·042	0·698	0·344		1½
7	Double N + minerals	1·085	0·696	0·389	Av. 0·392	1½
13	Double N + superphosphate + sulphate of potash	1·174	0·740	0·434		1½
17 + 18A	Double N following minerals	1·053	0·700	0·353		1½
6	Single N + minerals	1·119	0·651	0·469		½

If the plots are arranged according to the amount of ammonium salts transferred it is evident that the difference in the effect of rain on yield is not connected with this amount transferred. This can be seen from Table IV.

Table IV.

Plot	Amount of nitrogenous dressing transferred from autumn to spring	Difference in the effect of rain on yield (see Table III)	Mean grouped difference
	cwt.		
2B	0	−0·028	−0·028
5	0	−0·063	
17 + 18M	0	0 007	
6	½	0·468	0·468
10	1½	−0·008	0·280
11	1½	0·218	
12	1½	0·233	
14	1½	0·344	
7	1½	0·389	
13	1½	0·434	
17 +.18A	1½	0·353	
8	2½	0·177	0·177

The inferences to be drawn from Tables III and IV are very interesting. In Table III it is obvious that the plots which receive nitrogenous manures follow closely the results obtained by Fisher over the whole period, and thus correspond to their nitrogen abundance. Table IV indicates that the plots do not respond to the amount of ammonium salts transferred from the autumn to the spring, and hence it is clear that the difference in response to rain cannot be attributed to the differential manurial treatment.

It has been stated that plot 6 was the only plot which showed significant variation in the effect of rain throughout the year. Fig. 1 shows the average effect in bushels per acre of one additional inch of rain for plot 6 for both periods. It will be seen that an increased rainfall in the summer months of the early period is more detrimental to the yield than at any other time. This point is also brought out if similar curves are plotted for plots 2 B and 7, whereas during the total period of 65 years the greatest loss was usually ascribable to winter rain.

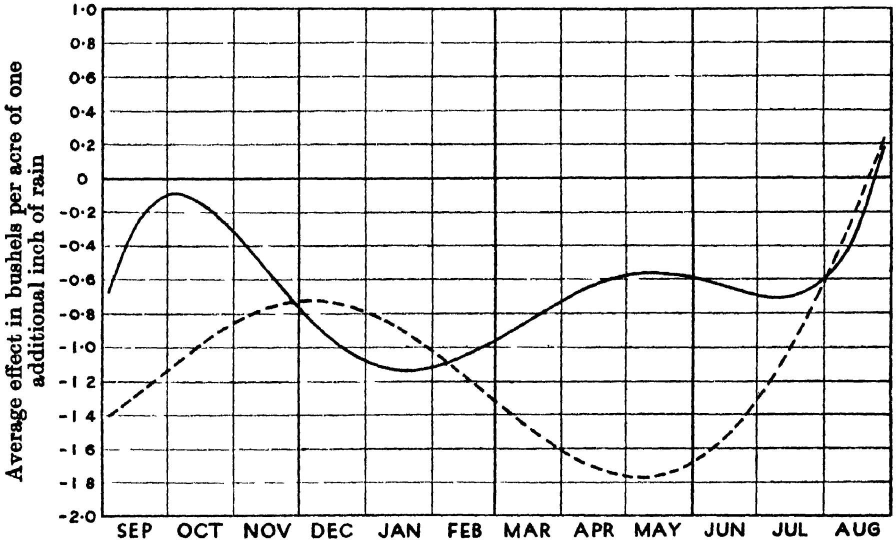

Fig. 1. Plot 6.

Continuous line ———— 1854–1918. - - - - Dotted line 1854–1877.

In comparing the two curves for plot 6, it is noticeable that more damage is caused by rainfall in the autumn, and in the months of March to July during the first 24 years; while there is no evidenee in support of the view that more damage is caused by winter rainfall for the period. The greatest difference is due to summer rainfall; this result is contrary to expectation, as it was thought that the heavy loss due to winter rainfall for the whole period might possibly be ascribed to the manurial treatment for the first 24 years.

COMPARISON OF SPRING AND AUTUMN DRESSINGS OF NITROGENOUS MANURES, 1879–1930.

As the previous work was essentially a comparison between autumn and spring dressings of nitrogenous manures, it was considered advisable to continue the investigations for a longer run of years. In this study the dependent variate is considered, not as the yield of one plot, but as the difference in yield between two plots, one of which receives 2 cwt. of nitrogenous manure in the autumn, the other receiving ½ cwt. in the autumn and the remainder, 1½ cwt., in the spring. The advantages obtained by taking this difference are numerous, the chief among which being that slow changes in yield caused by weed infestation, exhaustion of the soil, and the ravages caused by birds and insects are largely eliminated. Further, on several occasions Broadbalk wheat field has been partially fallowed, resulting in greatly increased yields in the years immediately following; and as fallowing has occurred more frequently in recent years, it would be very hard to get a satisfactory estimate of the yields of the different plots, so as to obtain a reliable series. By taking differences a figure is obtained representing the advantage or disadvantage of spring dressings over autumn applications, and although it is not claimed that this eliminates the effect of fallowing altogether, it is considered that the remaining trend will be reduced when polynomials of the third degree are fitted to the differences.

The problem was approached by the same method as before, cubic equations being fitted to the trend of each rainfall variate. A new determinant was formed from the sums of squares and products of the rainfall residuals, and this determinant was inverted as explained above.

The work proceeded through the several steps until the regression coefficients of difference in yield on the different rainfall variates were found. These were as follows, rainfall being measured in thousandths of an inch:

Table V.

Difference in yield between	Regression on					
	a'	b'	c'	d'	e'	f'
Plot 7 and plot 15	+0·0440591	+0·0150883	−0·0312224	−0·0488364	+0·0270855	−0·0421958
Plot 13 and plot 15	+0·0417156	+0·0055867	−0·0234240	−0·0433424	−0·0042797	−0·0268087
Plot 17 + 18A and plot 15	+0·0491304	−0·0130823	−0·0364184	−0·0518773	+0·0342302	−0·0259668
Average difference in yield	+0·0449684	+0·0025309	−0·0303549	−0·0480187	+0·0190120	−0·0316571

At this point it may be of interest to note that the regressions of the average difference in yield on the rain variates are the averages of the regressions of the individual differences. If to avoid confusion, the notation a', b', c', d', e', f' of the independent rainfall variates is changed to $x_1, x_2, x_3, x_4, x_5, x_6$, the polynomial fitting of the differences in yield, denoted by y', y'', y''', can now be expressed thus:

$$S_1' = Sy_r' = na_1,$$

$$S_2' = Sy_r'(n-r+1) = \frac{n(n+1)}{2!}b_1,$$

$$S_3' = Sy_r'\frac{(n-r+1)(n-r+2)}{2!} = \frac{n(n+1)(n+2)}{3!}c_1,$$

etc.,

$$S_1'' = Sy_r'' = na_2,$$

$$S_2'' = Sy_r''(n-r+1) = \frac{n(n+1)}{2!}b_2,$$

etc.,

$$S_1''' = Sy_r''' = na_3,$$

$$S_2''' = Sy_r'''(n-r+1) = \frac{n(n+1)}{2!}b_3,$$

whence

$$S_1' + S_1'' + S_1''' = S(y_r' + y_r'' + y_r''') = n(a_1 + a_2 + a_3),$$

therefore

$$S_1 = S\frac{(y_r' + y_r'' + y_r''')}{3} = \tfrac{1}{3}n(a_1 + a_2 + a_3).$$

Similarly

$$S_2 = \frac{1}{3}\frac{n(n+1)}{2!}\{b_1 + b_2 + b_3\}$$

and so on.

This shows that the constants of the polynomials can be averaged to give the polynomial constants of the average difference in yield, and hence the actual polynomials can also be averaged. The sums of products of yield residuals and residuals of x_1, etc., can likewise be averaged, for

$$S(x_1 - X_1)(y' - Y') = S(x_1 y') - nAA' - \frac{n(n^2-1)}{12}BB' - \ldots \text{etc.},$$

$$S(x_1 - X_1)(y'' - Y'') = S(x_1 y'') - nAA'' - \frac{n(n^2-1)}{12}BB'' - \ldots \text{etc.},$$

$$S(x_1 - X_1)(y''' - Y''') = S(x_1 y''') - nA''' - \frac{n(n^2-1)}{12}BB''' - \ldots \text{etc.}$$

Summing

$$S(x_1 - X_1)\{y' + y'' + y''' - Y' - Y'' - Y'''\}$$
$$= Sx_1(y' + y'' + y''') - nA(A' + A'' + A''')$$
$$- \frac{n(n^2-1)}{12} B(B' + B'' + B''') - \dots \text{etc.}$$

If both sides of this equation are divided by 3 the left-hand side becomes the sum of products of rainfall residuals and residuals of average difference in yield, while the right-hand side shows that this quantity is simply the average of the figures already obtained for the individual differences in yield. Hence the regression coefficients can be averaged.

For the sum of squares of residuals of the average difference in yield (necessary for the test of significance of seasonal variation) it was necessary to calculate $S(y'y'')$, $S(y''y''')$ and $S(y'y''')$, since

$$S\left(\frac{y' + y'' + y'''}{3}\right)^2 = \tfrac{1}{9}S(y'^2 + y''^2 + y'''^2) + \tfrac{2}{9}\{Sy'y'' + Sy''y''' + Sy'y'''\}.$$

This is the crude sum of squares, and must be reduced by subtracting the necessary corrections.

The regression curves for the differences in yield on rainfall (Figs. 2, 3 and 4) are very similar, and indicate that rainfall above the average in spring and summer is more detrimental to the plots which receive autumn dressings than to those manured in the spring. Winter rainfall has only a slight effect on the difference in yield. This result is more striking if the effect of a comparatively dry spring and summer is

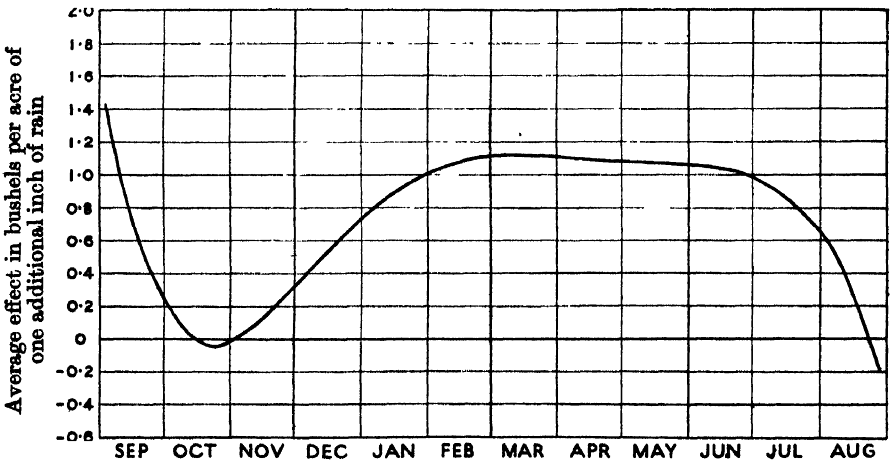

Fig. 2. Difference in yield plot 7 minus plot 15.

considered, as with these conditions the autumn dressings are more beneficial to the crop.

The curve for the difference in yield of plots 17 + 18A and 15 shows significant variation in the effect of rainfall throughout the year (Fig. 4).

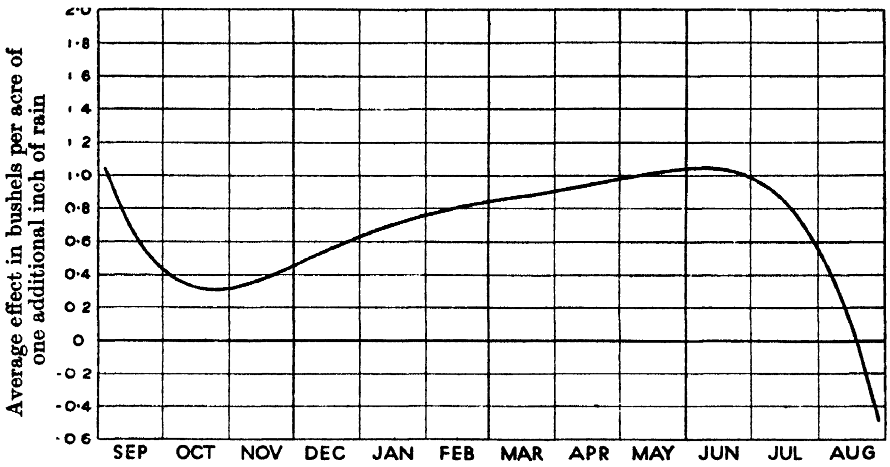

Fig. 3. Difference in yield plot 13 minus plot 15.

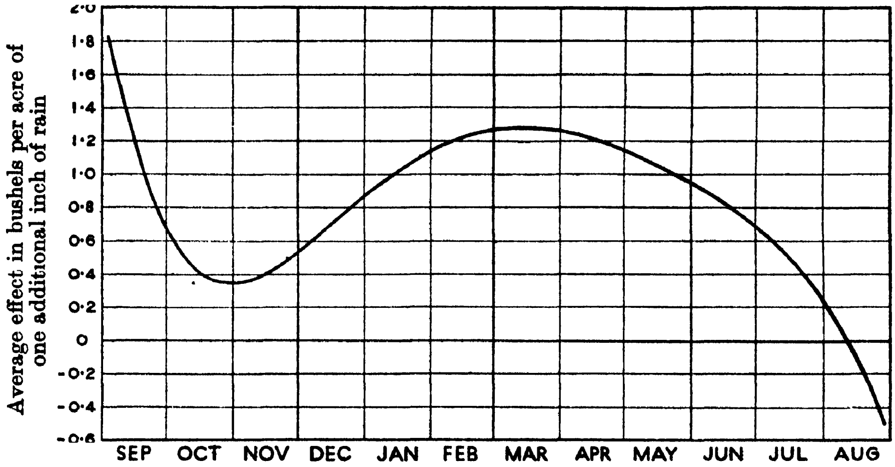

Fig. 4. Difference in yield plot 17 + 18A minus plot 15.

This result is not quite apparent in the other two differences, but the *z* test shows that both are near the level of significance. The cumulative evidence is found in the curve for the regression of the average difference in yield on rainfall (Fig. 5); this curve can be described as the average of the three curves, and it is interesting to note that it shows significant variation.

Table VI. *Tests of significance for variation in the effect of rain throughout the year.*

$n_1 =$ 5 degrees of freedom. Increase in variance due to introducing $b' \ldots f'$.
$n_2 = 42$ degrees of freedom. Variance due to deviations from regression function.
5 % point = 0·4455; 1 % point = 0·6247.

Difference in yield between	z
Plot 7 and plot 15	0·3600
Plot 13 and plot 15	0·2785
Plot 17 + 18A and plot 15	0·6838
Average difference in yield	0·5571

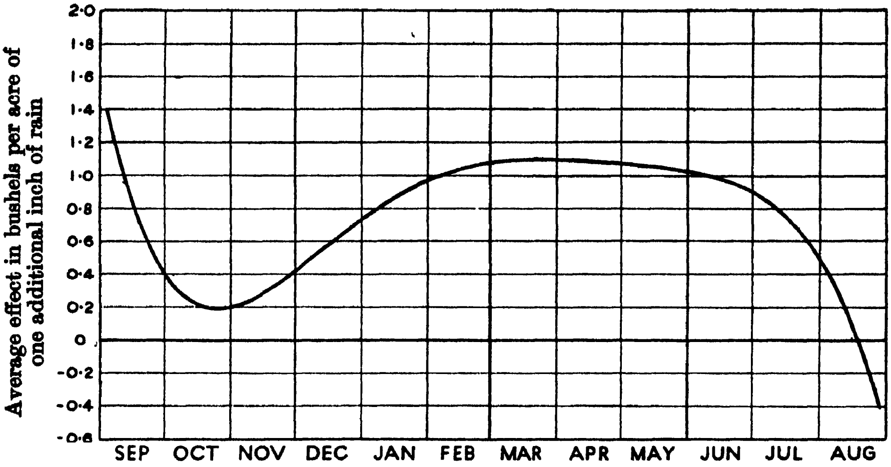

Fig. 5. Average of differences plot 7 minus plot 15, plot 13 minus plot 15, and plot 17 + 18A minus plot 15.

As the three curves tell somewhat the same story, the average curve can be taken as representing fairly the difference between autumn and spring dressings. Comparing the curve with the two curves already obtained for plot 6, it is at once obvious that there is confirmatory evidence of the result obtained above, *i.e.* that the greatest difference is due to summer rainfall, while little can be said about the winter effect.

Discussion.

It has been claimed that winter rainfall is responsible for washing out a large percentage of the nitrogenous manures applied in the autumn, and that in consequence plots which receive their dressings solely in the autumn have less nitrogen available in the spring than those which receive spring applications. Moreover, the plants take up most of their nitrogen in the spring and need it most when the rate of growth is greatest, usually about the month of May; hence the autumn-dressed plots would be at a serious disadvantage, and heavy rain at this period would be very detrimental to a crop already experiencing nitrogen shortage. On the other hand, spring dressing tends to produce greater yield; it promotes tillering if applied early, if applied late the effect is rather to increase the yield of grain. The theory that autumn-dressed wheat suffers especially from wet winters is, however, negatived by the results obtained in the curves. In a comparatively dry summer, the autumn dressing is equally as beneficial as the spring manuring; in fact, judging from the curves one might expect it to be at an advantage. This might be due to the fact that wheat is a deep-rooting plant, and it would require much rain to send the nitrogen far down into the soil. Further, plots manured in the autumn undoubtedly suffer to a certain extent from winter leaching; but it cannot be said that the crop on these plots is suffering from nitrogen starvation in the spring, as there is always residual nitrogen in the soil, even after the crop has been harvested.

Had the average rainfall distribution for the first 24 years been different from the distribution for the whole period, and if, in particular, the first 24 years had experienced heavier rainfall in the summer, then the two curves for plot 6 would not be quite comparable. An examination of the average distribution of rainfall throughout the year for the early period revealed that this is not the case, for during the summer months the distribution is almost exactly identical with that obtained by Fisher. There is, however, some evidence to show that, on the average, slightly less rain fell during the winter months of the early period.

Fisher's regression curves show a marked effect of winter rainfall, and this cannot be explained as due to the special conditions of the first 24 years. It would appear that his curves would be but little changed if, during the 65 years which he considered, the manurial treatments had been applied as they are now. The additional fact that spring-dressed plots are inferior to autumn-dressed plots in a dry summer, might perhaps be explained by the assumption that plants receiving all their nitrogen supply in the autumn have a better chance of establishing a good root system, which would place them at an advantage under these conditions.

The explanation of the results obtained by the curves is probably more complex than any that have been put forward above. No mention has been made of soil bacteria or the effect of rain on the soil itself, in fact there are several factors which might contribute to cause the results. These are subjects in which further research might be carried out by those qualified to do so.

SUMMARY.

The effect of rainfall on the wheat crop grown on Broadbalk has been investigated, special attention being paid to the time of application of the nitrogenous manures.

In the years 1854–77, when plots were manured solely in the autumn, the average decrease in yield caused by each additional inch of rain is greater than the average decrease from 1854–1918. The difference in this case cannot be ascribed to the change in manurial treatment.

The period of 24 years was too short to allow seasonal variation in the effect of rain to appear, as it was only possible to compare such seasonal variation with that of 1854–1918 in the case of one plot. In this case it was observed that summer rainfall was more detrimental to autumn-dressed than to spring-dressed plots; this result was confirmed by studies of the effect of rain on the difference in yield of two plots which differed only in the time of application of their manures.

ACKNOWLEDGMENT.

The writer would like to take this opportunity of recording his indebtedness to Dr R. A. Fisher, F.R.S., whose valuable time was so kindly given to help and encourage, and in whose laboratory this work was done. He is also indebted to Drs Wishart and Crowther, and to D. J. Watson, for help in discussing the results.

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Physiological Studies in Plant Nutrition.

III. Further Studies of the Effect of Potash Deficiency on the Rate of Respiration in Leaves of Barley.

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With seven Figures in the Text.

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INTRODUCTION.

IN a previous paper (5) the results of some experiments on the respiration and assimilation rates of leaves of barley grown under different manurial treatments were presented. It was there shown that, over a fairly wide range of manuring, phosphate has very little effect on the respiration rate, a deficiency of nitrate reduces respiratory activity, while potash deficiency increases it markedly. It was suggested (5, p. 154) that respiration rate must be a function of the amount of protoplasm, which in its turn must depend on the supply of nitrogen; hence, when this element is in the minimum, respiration rate, at least as ordinarily measured, is lowered. No evidence was found of a direct relationship between respiration rate and potash, and it was assumed that the increase in the rate under potash deficiency is due to the potash concentration determining the level at which other constituents of the cell are maintained.

[Annals of Botany, Vol. XLVI. No. CLXXXII. April, 1932.]

The present paper is the outcome of similar experiments performed in the summer of 1928, designed to elucidate further the relationship between potash and respiration.

EXPERIMENTAL PROCEDURE.

The experimental procedure was similar to that of the previous year; barley of the variety Plumage Archer was used, and the plants were grown three in a pot in sand culture as before. Four nutrient solutions were employed, which differed only in the amount of potassium sulphate they contained; the following are the weights of the pure salts given to each pot in the 'fully manured' series:

Na_2HPO_4	2·52 grm.
$NaNO_3$	9·1 „
K_2SO_4	1·85 „
$CaCl_2$	0·37 „
$MgSO_4$	0·61 „

This solution is identical with the one given to the 'fully manured' series of 1927. The plants grown under these conditions will subsequently be referred to as 'Series A'. Series 'B' and 'C' differed in having only one-fifth and one-tenth respectively of the amount of K_2SO_4 given to Series A (0·37 and 0·185 grm.), while Series 'D' was grown without the addition of any potash, except such as was present in the tap-water used for watering the pots.

The seed, previously sterilised with formalin, was sown on May 2, and the nutrients applied on May 7; germination began on May 10, and was practically completed by May 12.

The technique used in 1928 was almost identical with that of 1927, but instead of working in a constant temperature room, the katharometer and leaf chamber were housed in a large thermostat.

A higher temperature was used than in the previous year; the maximum variation between different experiments was 2° C., and the observed rates have been given a slight correction so as to express them all at the approximate mean of 24° C. Owing to the higher temperature, the respiration rates were at a considerably higher level than those of the previous year, and therefore the 'zero drift' of the instrument was of relatively less importance; the maximum error in the respiration rate which is ascribable to such a cause cannot be greater than 2 per cent.

Respiration rates of one particular leaf from each of the four series were determined each week, the determinations occupying four successive days. The first determinations were made on the first leaves from May 23–26, but owing to an accident the results from Series B and D were untrustworthy. The succeeding eleven weeks provided respiration rates from the successive young leaves on the main stem as they reached maturity, from the second to the tenth. Because of differences in the rates of emergence from weekly intervals, the seventh leaf was omitted, while two successive determinations were made on the ninth. The last three determinations were made on the tenth leaf, and show the senescent changes thereof.

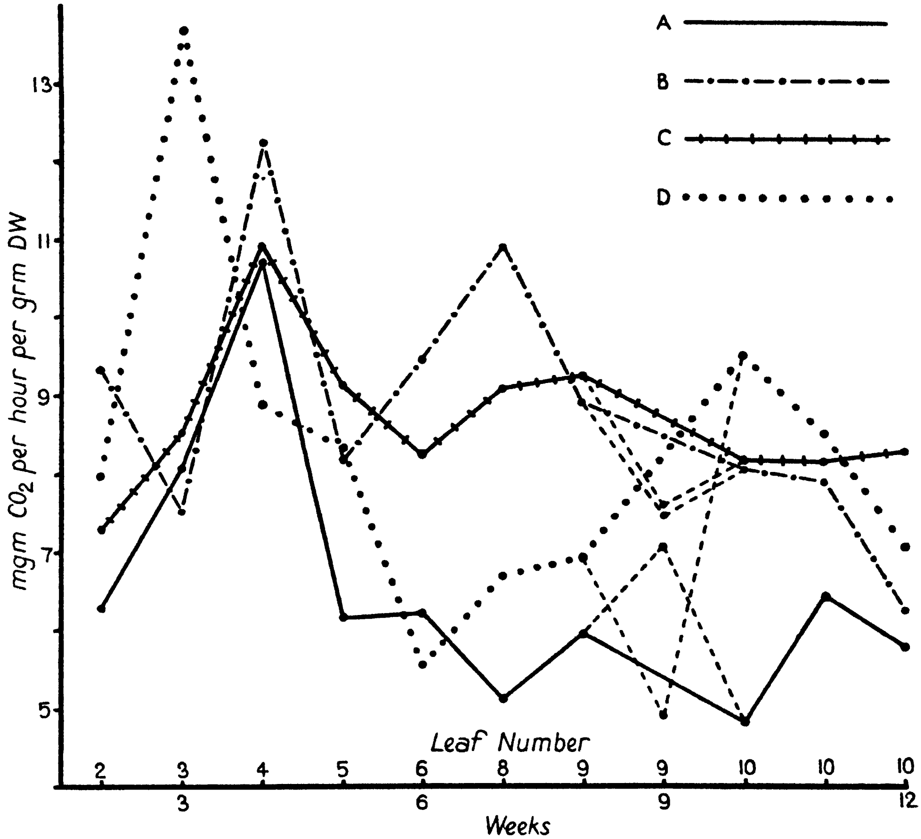

FIG. 1. Graph showing rates of respiration, on a dry weight basis, of the successive leaves from the four potash series.

In actual practice each observed rate was that given by at least two leaves cut from different plants, strips about 3½ in. long being taken from the central region. The cut ends of the leaves dipped into a film of water.

EXPERIMENTAL RESULTS.

Respiration Rate and Potash Deficiency.

In Table I are presented the rates of respiration, corrected to the approximately mean temperature o 24° C., and expressed in terms of dry weight and leaf area. These values are plotted in Figs. 1–4. In Figs. 1 and 2, respiration rate is plotted for the four potash concentrations against time, whereas in Figs. 3 and 4 the corresponding weekly rates from the four series are plotted against the amount of potash applied in the nutrient solutions. The maximum respiration rate in Series A, B, and C was attained at the fourth leaf, while in D it was reached at the third; after this maximum, B and C showed a gradual decline in rate, but in A and D a more sudden drop to a considerably lower value occurred, followed in D by a secondary rise in the later formed leaves. Manurial differences are very pronounced after about the fourth leaf; on a dry weight basis (Fig. 1) B and C are not very different, but are considerably higher than the fully manured series. At very low potash concentrations respiration rate is again lowered, so that D takes an intermediate place between B and C on the one hand, and A on the other. On a leaf area basis the percentage differences due to manuring are very much increased, and in the middle period of growth the four curves become widely spaced, the order being B (highest),

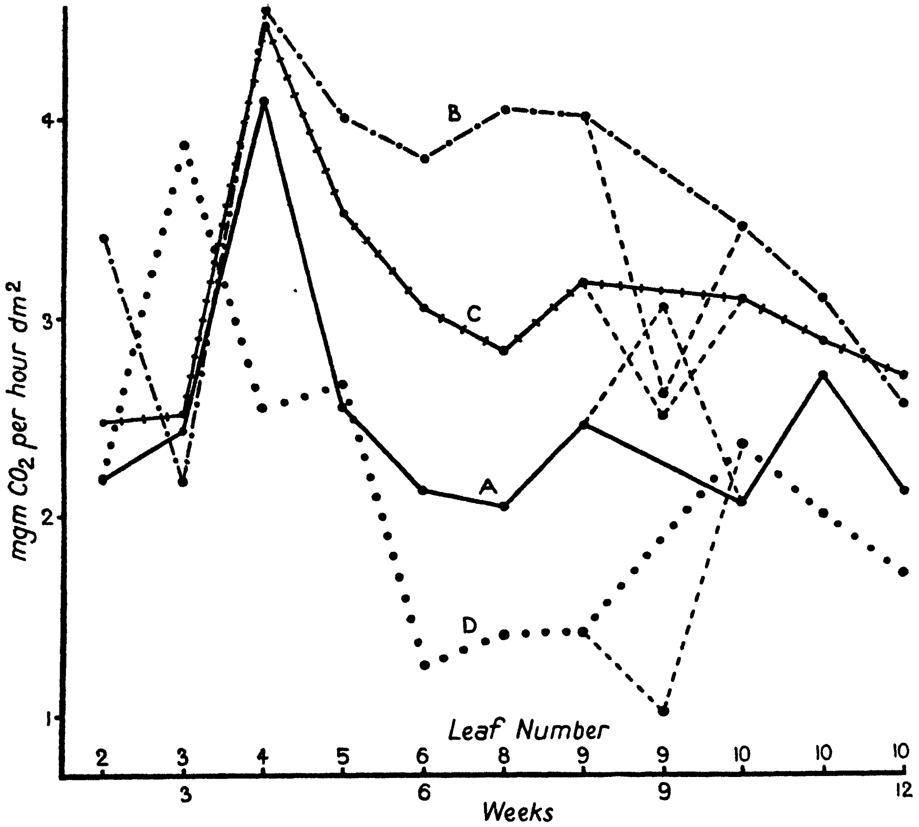

FIG. 2. Graph showing rates of respiration, on a leaf area basis, of the successive leaves from the four potash series.

C, A, and D. These differences are considerably diminished in the last two leaves.

TABLE I.

Rates of Respiration.

Dry weight basis gives mgms. CO_2 per hour per grm. dry weight.
Leaf area " " " " " " per dm^2. leaf surface.

	A.		B.		C.		D.	
Leaf No.	Dry Weight basis.	Leaf Area basis.	Dry Weight basis.	Leaf Area basis.	Dry Weight basis.	Leaf Area basis.	Dry Weight basis.	Leaf Area basis.
1	5·62	1·585	—	—	6·60	2·008	—	—
2	6·29	2·185	9·33	3·402	7·28	2·471	7·98	2·183
3	8·07	2·426	7·52	2·165	8·50	2·507	13·66	3·869
4	10·72	4·092	12·23	4·547	10·93	4·477	8·87	2·541
5	6·15	2·540	8·16	3·998	9·11	3·517	8·31	2·658
6	6·21	2·115	9·46	3·793	8·22	3·043	5·53	1·240
8	5·10	2·033	10·90	4·039	9·08	2·820	6·68	1·398
9	5·94	2·447	8·90	4·025	9·25	3·169	6·93	1·415
9	7·08	3·057	7·44	2·611	7·58	2·495	4·89	1·007
10	4·81	2·053	8·06	3·449	8·17	3·088	9·52	2·360
10	6·46	2·712	7·92	3·103	8·17	2·883	8·51	2·015
10	5·79	2·128	6·26	2·574	8·31	2·714	7·09	1·712

As the amount of potash supplied to the plant is decreased, so the respiration rate of the leaves, however it is expressed, increases until a maximum value is reached, after which further decrease in potash supply is accompanied by decrease in respiration rate.

The figures obtained have been subjected to the Analysis of Variance, the results of which are given below:

TABLE II.

Dry Weight Basis.

Variance due to—	Degrees of Freedom.	Sum of Squares.	Mean Square.	z.	5 %.	1 %.
Manuring	3	31·53804	10·51268	0·767	0·536	0·753
Leaf Number	10	51·58761	5·158761	0·411	0·389	0·550
Remainder	30	68·01263	2·2670877			
Total	43	151·13828				

Leaf Area Basis.

Variance due to—	Degrees of Freedom.	Sum of Squares.	Mean Square.	z.	5 %.	1 %.
Manuring	3	11·992200	3·997400	1·194	0·536	0·753
Leaf number	10	8·324634	0·8324634	0·409	0·389	0·550
Remainder	30	11·018467	0·36728223			
Total	43	31·335301				

Thus on either basis there is a real effect due to treatment, as well as a real effect due to age, or leaf number. The differences between the means of the four treatments are given below, together with the appropriate standard error :

	Differences of means.	
Comparison	Dry Weight basis.	Leaf Area basis.
A–B	−2·142	−0·902
A–C	−1·998	−0·491
A–D	−1·396	+0·490
B–C	+0·144	+0·411
B–D	+0·746	+1·392
C–D	+0·603	+0·981
Standard error	0·642	0·258

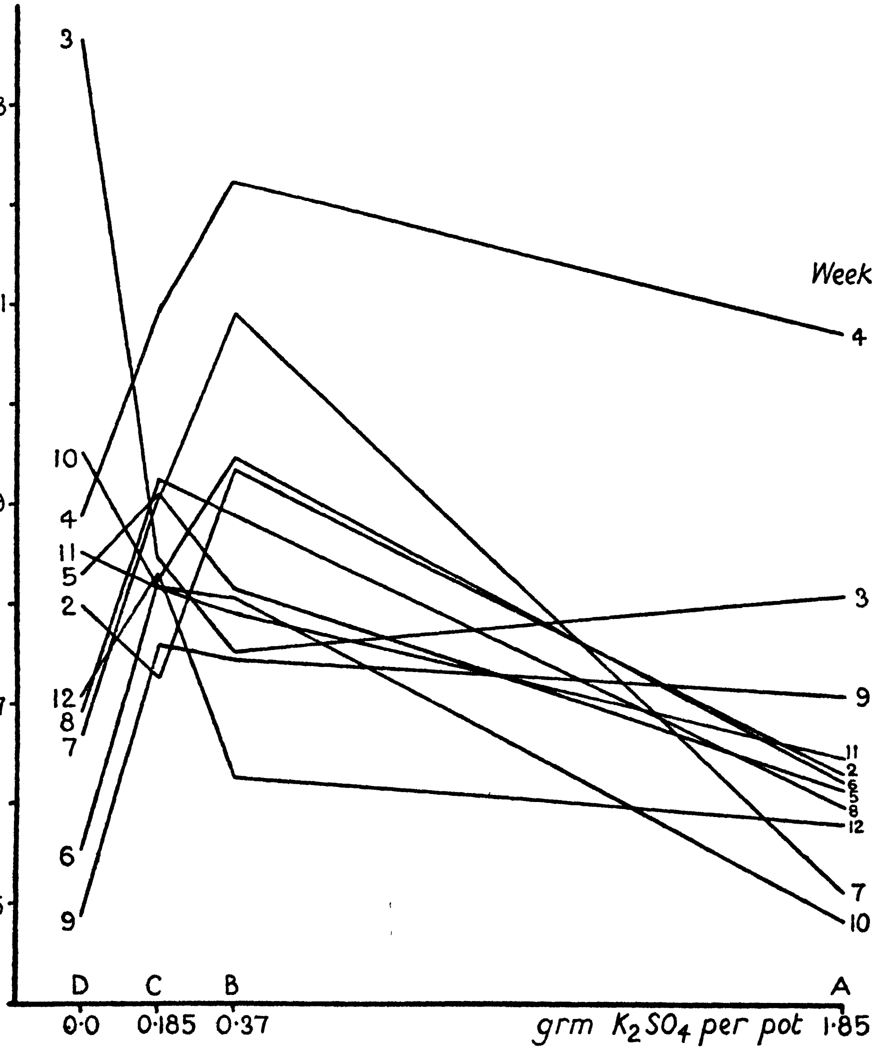

FIG. 3. Graph showing rates of respiration, on a dry weight basis, of the successive leaves, plotted against the amount of potash applied.

The differences greater than twice their standard error, those with a probability of significance greater than twenty to one, are shown in heavy type. On a dry weight basis all that is definitely proven is that A has a lower

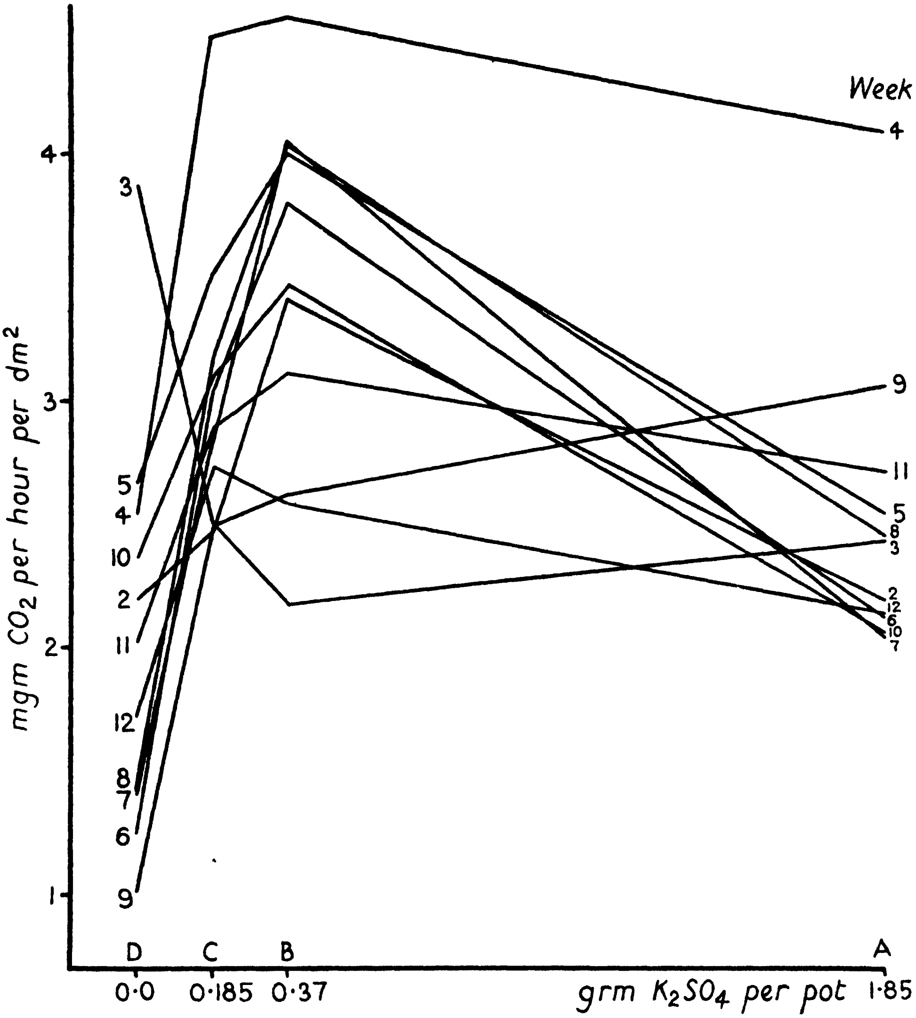

FIG 4. Graph showing rates of respiration, on a leaf area basis, of the successive leaves, plotted against the amount of potash applied.

respiration rate than any of the deficient series, while on a leaf area basis the rate in D is shown to be lower than that in B and C, and that in A also to be lower than that in B. It is desirable also to demonstrate that on a dry weight basis the rate at very low potash concentrations (D) is lower than that at medium concentrations (B and C). A glance at Fig. 1 abundantly demonstrates that the interaction between potash concentration and leaf number must be very considerable, the four curves being far from parallel and similarly situated; and all the variance due to this cause has been included in experimental error, which must therefore be an exaggerated estimate. It is also clear that the four values obtained from the third leaf must contribute an excessive amount to this estimate, seeing that the value for D is there much higher than are those of the other three series. It is unlikely that this is due to chance causes, but in all probability a real interaction is indicated. If the respiration rate rises as potash deficiency becomes more and more acute, Series D may be expected to show the effects of deficiency earlier than the others, and hence it seems very probable that the high value in the third leaf is real, and should not contribute an excessive amount to the estimate of experimental error. Better justification for this omission will appear later (p. 385).

An Analysis of Variance on the respiration rates (dry weight basis), omitting the four values at the third leaf, then, gives the following results:

TABLE III.

Dry Weight Basis.

(Omitting Third Leaf.)

Variance due to—	Degrees of Freedom.	Sum of Squares.	Mean Square.	*z*.	5 %.	1 %.
Manuring	3	37·31081	12·43694	1·090	0·543	0·763
Leaf number	9	42·31339	4·701488	0·603	0·408	0·577
Remainder	27	37·98459	1·406836			
Total	39	117·60879				

It is seen that by this omission the mean square ascribed to error is considerably lowered, and the significance of the *z* values much increased; the standard error shows also that, except in the earliest and last leaves, respiration rate is significantly lower in Series D than in either B or C:

Comparison.	Differences of Means.	Standard Error.
A–B	−2·411	±0·530
A–C	−2·155	
A–D	−0·976	
B–C	+0·256	
B–D	+1·435	
C–D	+1·179	

Another apparent effect of potash starvation—a lowering of the temperature coefficient of respiration—may conveniently be mentioned here, though it is not desired to press a result derived from such few data. The potash deficient series of 1927 corresponded very nearly in manuring to Series C of 1928; the mean temperature used in the former year was 17° C., and in the latter 24°. Assuming the differences in the mean rates of respiration in corresponding manurial series between the two years to be due entirely to temperature, the value of Q_{10} in the fully manured plants is 3·02, while that in the potash deficient series is 2·43. Two actual estimations of the temperature coefficient in Series A and D were made over the range corresponding to the two years' experiments, using the sixth and the eighth leaf respectively. For the sixth leaf, the value of Q_{10} in A was 2·20, and in D 1·56; while for the eighth that of A was 1·57, and of D 1·235.

Water Relationships of Leaves, and their Bearing on the Choice of a Basis for the Expression of Respiration Rate.

In Table IV are given, for the four series of leaves, the values obtained for water content expressed as a percentage of the dry weight, and for the ratio of dry weight to leaf area.[1]

TABLE IV.

	A.		B.	
Leaf No.	$\frac{\text{Dry Weight}^*}{\text{Leaf Area}}$.	$\frac{H_2O \times 100}{\text{Dry Weight}}$.	$\frac{\text{Dry Weight}^*}{\text{Leaf Area}}$.	$\frac{H_2O \times 100}{\text{Dry Weight}}$.
1	30·4	577	—	—
2	36·0	570	35·9	586
3	31·0	657	31·1	741
4	40·8	573	38·5	636
5	41·3	498	49·0	476
6	34·1	577	40·1	558
8	39·9	516	37·0	543
9	41·2	502	45·2	475
9	43·2	357	35·1	411
10	42·6	236	42·8	217
10	42·0	248	39·2	258
10	36·7	279	41·1	252

	C.		D.	
Leaf No.	$\frac{\text{Dry Weight}^*}{\text{Leaf Area}}$.	$\frac{H_2O \times 100}{\text{Dry Weight}}$.	$\frac{\text{Dry Weight}^*}{\text{Leaf Area}}$.	$\frac{H_2O \times 100}{\text{Dry Weight}}$.
1	31·4	585	21·6	841
2	33·4	604	29·2	624
3	32·7	670	31·8	716
4	43·6	553	29·9	827
5	38·6	608	32·0	782
6	37·0	598	22·4	1053
8	31·1	741	20·9	1119
9	34·3	448	20·4	1078
9	32·9	371	20·6	783
10	37·8	290	24·8	479
10	35·3	277	23·7	457
10	32·7	307	24·1	551

* Weight in cgms. per dm^2. Leaf Surface.

If these figures are examined by means of the Analysis of Variance, it is found that the manurial differences in both characteristics are very much too great to be accounted for by chance; in water content, the value of z is 1·586 (1 per cent. = 0·753), while in the ratio of dry weight to leaf area it is 1·720 (1 per cent. = 0·753). If the mean values of the series are examined among themselves by means of the appropriate standard error the following results are obtained:

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[1] The legend of Table I in the previous paper (5) should have read *centigrams* per sq dm., not mgms.

TABLE V.

Water Content.	Differences of Means.
D–C	$+272·9 \pm 43·2$
C–B	$+28·5 \pm 43·2$
B–A	$+12·7 \pm 43·2$

Dry Weight: Leaf Area.	Differences of Means.
D–C	$-9·96 \pm 1·65$
C–B	$-4·15 \pm 1·65$
B–A	$+0·56 \pm 1·65$

It will be seen that in water content A, B, and C have not been shown to differ significantly from one another, but that D is very significantly higher than the others; in the ratio of dry weight to leaf area A and B show no difference, but C is significantly below them, and D again very significantly below C. There can be no doubt that the difference in water content between C and either A or B is real, but that this reality is hidden by the use of a much exaggerated estimate of experimental error. As can be seen from the Table, or from Fig. 5, there is a very different behaviour in time between D and the other series and, owing to the fact that replicated values were not obtained, the variance due to this has been included in that ascribed to experimental error. This leads to a standard deviation as high as 18·5 per cent. of the mean value in this characteristic, while in the ratio of dry weight to leaf area the corresponding standard deviation is only 11·1 per cent. of the general mean.

In the previous publication it was pointed out that a high negative correlation exists in time between (1) the ratio of the water content in one manurial series to that in another, and (2) the ratio between the dry weights of unit leaf area in the same two manurial series. A diagram was there given of this interrelationship in nitrate deficient and fully manured plants, and a similar diagram is presented here (Fig. 6) for the potash Series D and A, except that the logs of the ratio values are plotted instead of the values themselves, in order to bring out the correlation more clearly. The correlation coefficient between the actual values is −0·918 (1 per cent. = 0·708). The middle curve is obtained by adding together corresponding points on the other two, and represents the log of the ratio of the weight of water per unit leaf area in D to the weight of water per unit leaf area in A. As before, this ratio is found to be much more constant than the corresponding ratio of water content on a dry weight basis, but yet to deviate definitely if slightly from the zero line in the same direction. In Fig. 5 the weight of water per unit leaf area in these two series is shown, together

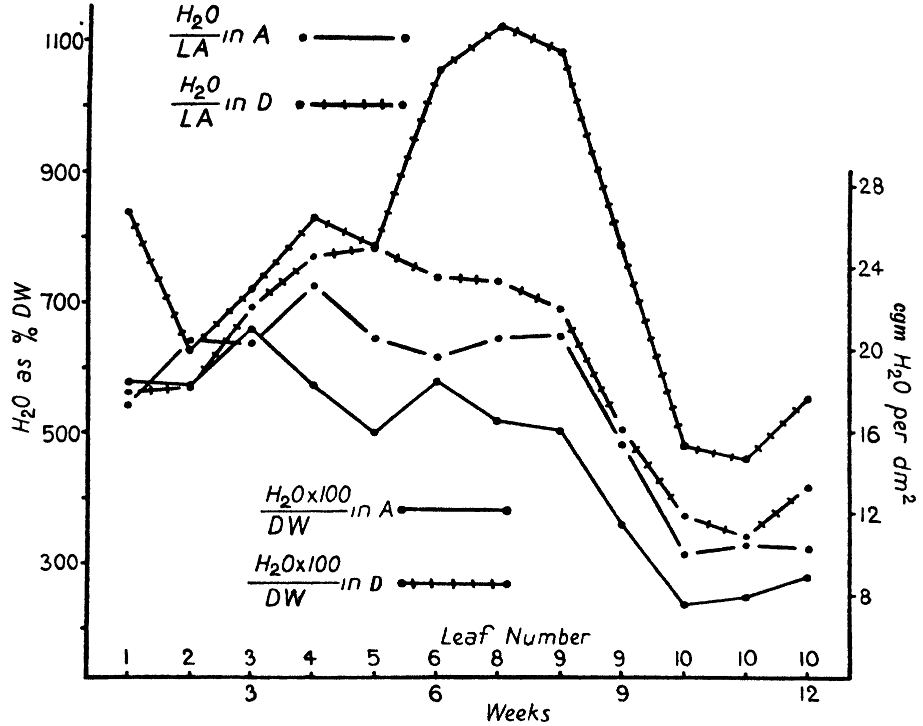

FIG. 5. Graph showing water content of the successive leaves in Series A and D, expressed in terms of both dry weight and leaf area.

with the water content expressed as a percentage of dry weight, for comparison.

The Analysis of Variance of this characteristic shows that, in spite of a considerably lower experimental error than in the ratio of dry weight to leaf area even (below 8·0 per cent. of the mean), the differences between the manurial treatments are so small that D is the only series differing significantly from the others; the difference between D and A is not so great as three times its standard error:

Water : Leaf Area.	Differences of Means.
D–C	+1845 ± 676·4
C–B	−689 ± 676·4
B–A	+811 ± 676·4

It appears, then, that under a type of mineral salt deficiency that leads to a divergence from the normal in the ratio of water to dry weight, the amount of water per unit leaf area shows a corresponding and real, though much less pronounced, deviation. It seems fairly clear that the differences observed in the ratio of dry weight to leaf area between manurial treatments can be largely accounted for by a contraction or expansion of leaf surface due to an abnormally small or great amount of water respectively. What is not so clear at first sight is that, since real and small differences do exist in the amount of water per unit area of leaves from plants treated differently, it is quite possible that practically all the differences found between treatments in the ratio of dry weight to leaf area may be attributable to this cause.

Thoday (12), working with *Helianthus*, was the first to show that leaves will expand and contract with gain or loss of water, and some unpublished data of the present author show that this is perfectly true of leaves of barley, both under conditions of complete nutrition, and of deficiency in potash. There is no reason to suppose that leaves from barley deficient in nitrate or phosphate behave differently.

There is also little reason to expect that an expansion of leaf surface, due to an increase in water content, would not be reflected in a similar expansion in leaf thickness, and indeed, Bachmann (1) has recorded considerable changes in this dimension due to water variation. If such an expansion affects all three dimensions of the leaf proportionally, and the expanded area be x times the unexpanded, then the expanded leaf volume will be $x^{1.5}$ times the unexpanded. If a similar relation[1] can be shown to hold between the various manurial types, it may then be assumed that dry weight per unit leaf area, when measured at constant water content, is unaffected by manurial treatment; since it appears unlikely that the relative expansion in the three dimensions, due to water, will be appreciably affected by the manurial treatment. For the leaves under consideration, those of the four manurial types of 1927 discussed in the previous paper, and the potash deficient series of 1928, the leaf surface representing a given dry weight is known; the leaf volume representing the same dry weight is more difficult to estimate, but if we neglect intercellular spaces, the actual weight of water associated with that dry weight will be approximately proportional to the required volume of the leaf. Another approximation will be given by the ratio of fresh weight to dry weight, seeing that the density of the living leaf substance cannot differ markedly from unity. Since a considerable portion of the dry weight must exist in the living leaf in solution, it would seem that the best estimate of volume per unit dry weight, from the data available, is one intermediate between the estimate based on water alone and that based on fresh weight.

The leaf data from the two years' experiments have been examined from this point of view. The various comparisons indicate that in general the value of the exponent is greater than unity, and therefore that there is a real expansion in thickness accompanying an expansion in area. Values both above and below 1·5 have been obtained. It appears, then, that if the ratio of water to leaf volume were plotted for the different manurial treatments, the curves would be still more nearly coincident than are those of the ratio of water to leaf area; and hence that, if a complete correction were applied to the ratio of dry weight to leaf area for water content, the differences between the manurial curves would become very markedly less, and almost, in fact, disappear.

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[1] $\frac{\text{Vol. of unit dry wt. in treatment } x}{\text{,,} \quad \text{,,} \quad \text{,,} \quad y} = \left(\frac{\text{Area of unit dry wt. in } x}{\text{,,} \quad \text{,,} \quad y}\right)^{1.5}.$

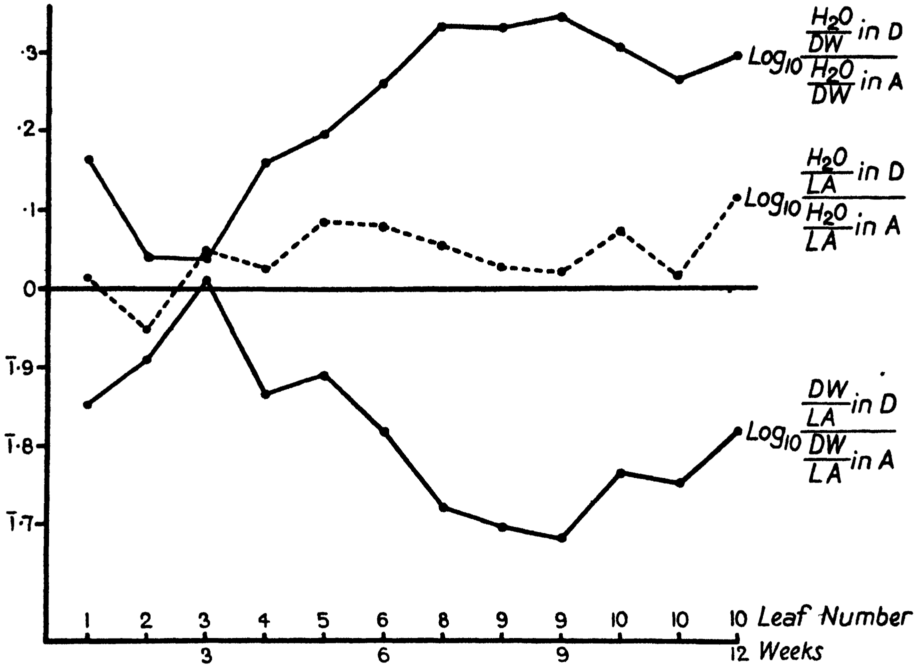

FIG 6. Graph showing various water relationships in the leaves of Series A and D. For explanation see text.

Differences between the successive leaves of one plant as they emerge cannot be accounted for in the same way. Water content shows a considerable and progressive change from leaf to leaf, whereas dry weight to leaf area fluctuates more nearly about a mean value, indicating that could leaf area be measured at a uniform water content in the successive leaves, the ratio of dry weight to area would show a marked fall in the later leaves, as against the earlier. The conclusion is foreshadowed by the fact that when water content is expressed in the form of the ratio of the weight of water to leaf area the percentage difference between treatments is much diminished, while that between the successive leaves is almost unchanged.

From these considerations, the conclusion emerges that deviations in the ratio of dry weight to leaf area due to manurial treatment are mainly of a different nature from corresponding deviations between the successive leaves of the plant. The former have very little real existence apart from water differences, whereas the latter must represent differences of leaf structure.

The preceding discussion establishes that almost all the divergence in the ratio of dry weight to leaf area brought about by the manurial differences studied is due to the extent to which the leaves are inflated with water. Hence the large differences between the curves of respiration of successive leaves, as expressed on a dry weight (Fig. 2) and a leaf area (Fig. 4) basis, are attributable to differences in water content resulting from manurial treatment. Since it is desirable to exclude from the respiration relations the action of a second variable factor affected by manuring, namely water content, it would seem preferable to express respiration results for comparative purposes in terms of dry weight. A and B show no real difference in water content; similarly the general relationship between curves A and B is almost identical in Figs. 1 and 2; on the dry weight basis, the mean respiration rate of B is 132·4 per cent. of that of A, and on a leaf area basis 135·9 per cent. C has a higher water content, and the leaf expansion in this series leads to a drop in the respiration rate on a leaf area basis to a position intermediate between A and B; while the very high water content of D leads similarly to a considerable relative drop, bringing it well below that of the fully manured series. Thus, if we consider the value of the mean respiration rate in C in terms of that in A, the ratio of this value on the dry weight basis to that on the leaf area basis is 1·0906; the corresponding ratio of the water contents in terms of dry weight, in C and A, is 1·0905. Similarly, D and A give the corresponding values 1·503 and 1·689.

Potash Content of Leaves.

The leaves used in these experiments were subsequently analysed for potash content,[1] and the results are presented in Table VI. The differences in potash content between leaves from Series A and those from the other series is very marked; B, C, and D are much more nearly alike in the amounts they contain. Thus, if we consider the percentage of K_2O in the dry weight of the leaves, the mean values between determinations 2 and 12 for the Series A, B, C, and D, are 2·347, 0·759, 0·671, and 0·539 respectively; even neglecting the earlier leaves, and considering the last four determinations after the minimum in potash concentration had been reached, the corresponding values are 2·718, 0·469, 0·478, and 0·341. The values obtained for potash concentration within the leaf indicate very strongly that this is much higher in the earliest and latest formed leaves

[1] The author again wishes to thank Dr. Janet W. Brown for undertaking these analyses.

than in the intermediate ones, but in the case of Series A the curve shows some marked irregularities. These irregularities appear to correspond largely with periods of heavy rainfall, and the correlation with rainfall has therefore been examined. In order to do this, a parabola was fitted to the curve of potash concentration in the successive leaves, and the deviations from this parabola correlated with deviations from the parabola similarly fitted to the rainfall curve, the rainfall chosen being the amount which fell during the week preceding the day on which the leaves were cut from the plant. This method is equivalent to correlating potash concentration with rainfall, eliminating from the total correlation that which may be due to the first and second powers of time, i.e. the general shape of the potash concentration curve. The coefficient obtained is negative and high, −0·745, a value which, considering the appropriate number of degrees of freedom, has a probability of significance of approximately 50 : 1.

TABLE VI.

Per cent. K_2O *on Dry Weight Basis.*

Leaf No.	A.	B.	C.	D.
1	2·47	—	1·278	1·658
2	3·53	2·264	1·449	1·396
3	2·97	1·153	1·143	0·950
4	1·61	0·968	0·888	0·425
5	1·66	0·538	0.473	0·882
6	1·71	0·821	0·509	0·246
8	1·96	0·374	0·359	0·478
9	1·51	0·353	0·649	0·186
9	2·21	0·656	0·761	0·340
10	3·68	0·587	0·576	0·412
10	1·74	0·389	0·370	0·534
10	3·24	0·242	0·204	0·078

The values for rainfall used in this correlation may appear somewhat arbitrary, but this particular measure was not chosen because it gave a higher correlation coefficient than others. An ideal estimate of 'rainfall' would probably be a weighted one in which recent rain was given a higher value than rain which had fallen some days previously, from the effects of which the leaf has presumably had time partially to recover; and in fact, had such an estimate been used in the present instance, a considerably higher correlation would have been obtained.

The data from the fully manured series of 1927, when analysed in a like manner, also give a high and negative correlation coefficient, −0·565. This has a probability of significance greater than 10 : 1 but less than 20 : 1, and, though this is not usually regarded as 'significant', it points in the same direction as the 1928 data. It is interesting to note that the mean rainfall value in 1927 (0·934 in.) was much *higher*, and the rain fell more uniformly, than in 1928 (0·544 in.), while the potash content in 1927 (mean = 6·92 mgms. per sq. dm.) was considerably and consistently *lower* than in 1928 (8·91 mgms.), which is consistent with the relation indicated by the above correlations.

Series B, C, and D do not show a similar variation with rainfall.

Mann and Wallace (8) have shown that, in the case of apple trees, potash may be washed out of living leaves by means of running water; so easily, in fact, is this done that a similar leaching occurs in the field during rainy periods. Other workers have reported somewhat similar results. The above data taken collectively indicate strongly that the same phenomenon occurs in nature in the case of leaves of barley. This is true of plants which have been given an ample supply of potash, but where this element is deficient and the internal concentration low, the absence of any correlation with rainfall would appear to show that the potash present is less readily removed and has not the comparatively loose association with the tissues which seems characteristic of much of the potash in plants having a high internal concentration.

GENERAL DISCUSSION.

As was found for the fully manured and potash deficient plants in 1927, there is again no demonstrable relationship in any of the four present series between the potash concentration and the respiration rates of the successive leaves with the exception of Series D. The interrelationship of these two variables between the four series also is apparently not of a simple nature, seeing that potash content drops greatly from A to B, and very slightly from B to D, whereas respiration rises from A to B, and falls markedly from C to D.

An explanation of these results may be sought in the amount of carbohydrate to be found in the leaves of plants given different amounts of potash, but, as has been shown (5), over a period in the history of partially deficient plants when the carbon assimilation rate is markedly subnormal the respiration rate of the same leaves is markedly supernormal. Furthermore, unpublished work by F. G. Gregory indicates that under moderate deficiency the leaves of barley do not have a higher sugar concentration than normal, but the reverse, and these results are supported by other workers (7, 10). On the other hand, it appears probable that the lowering of respiration rate between Series C and D is due to the low concentration of carbohydrate material, seeing that the assimilation rate (Fig. 7) in Series D was very subnormal throughout almost the whole life-history of the plants. In this connexion, the correlation coefficients between assimilation rate and respiration rate in the four series are interesting. Below are given the total correlations, and also the correlations after eliminating the general effect of time:

Series.	Total Correlation.	Partial Correlation.
A	+0·0426	−0·166
B	+0·1959	−0·011
C	+0·2356	+0·164
D	+0·8724	+0·827
1 % point	0·735	0·765

It is seen that these correlations are quite insignificant in manurings A, B, and C, but highly significant in the case of the totally starved plants, in which the curve of respiration rate reproduces closely that of assimilation rate (Figs. 2 and 7). The small positive partial correlation in Series C may be an indication that also at this level of potash manuring respiration rate is partially dependent on the assimilatory capacity of the leaves, and presumably, therefore, on the carbohydrate concentration, since the respiration rate was observed shortly after a period of assimilation.

The closeness of the agreement between respiration and assimilation rates in Series D, both being measured in terms of leaf area, is worth examining more closely. At this level of manuring, and at this level only, a high and significant positive correlation is found between respiration rate and potash content for the successive leaves. The coefficients (on a leaf area basis) are as follows:

Series.	Correlation.	
A	−0·3901	5 % = 0·602
B	−0·2169	
C	−0·0858	
D	+0·6467	

It was shown in the previous publication (5, p. 153) that a similar high correlation in potash-deficient plants was found to be spurious, and to be entirely dependent on the dry weight per unit area of the leaves. Here also the relationship is a spurious one, as may be seen by eliminating from the total correlation that part which is due to assimilation rate. The results are illuminating. In Table VII are presented for the four manurial series the partial correlation coefficients between each pair of the three variates, respiration rate (*r*), assimilation rate (*a*), and internal potash concentration (*k*), all being measured in terms of leaf-area. From the total correlation between each pair has been eliminated that due to the third variate:

TABLE VII.

Series	$r_{ar\cdot k}$	$r_{ak\cdot r}$	$r_{rk\cdot a}$
A	−0·325	−0·163	−0·389
B	+0·199	+0·462	−0·067
C	+0·317	+0·868	−0·234
D	+0·779	+0·655	−0·203
	5 % = 0·632	1 % = 0·765	

From column 2 it is clear that assimilation rate is independent of potash concentration when this is high (A); but that the partial coefficient rapidly rises with the degree of starvation, being highly significant in C and D. Column 1 shows with increasing deficiency a similar though more gradual rise in the coefficient between assimilation and respiration rates; while column 3 shows that the high positive total correlation between respiration rate and potash content in D has entirely disappeared, and that at no level of potash manuring is there any appreciable correlation between the two; what correlation there is appears to be negative. The conclusion is, therefore, that under extreme starvation (D) assimilation rate is dependent on the potash content of the leaves, and that respiration rate is in its

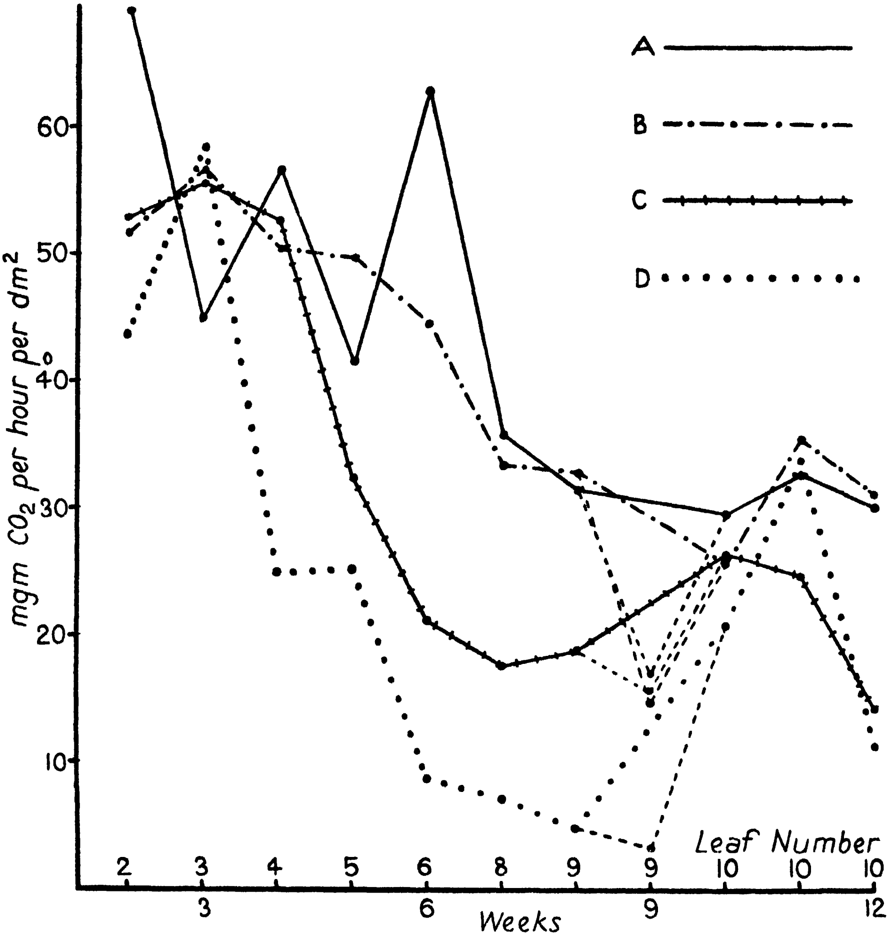

FIG. 7. Graph showing rates of assimilation, on a leaf area basis, of the successive leaves from the four potash series. Temperature, 24° C.; light intensity, 15,250 metre candles excess CO_2.

turn dependent on the assimilation rate. A similar condition, though less marked, is probably found in Series C; but where the potash supply is ample (A) no appreciable correlation exists between any two of the three variates.

It is not intended to discuss here the relationship between assimilation and potash except in its bearing on respiration rate. At the beginning and at the end of the life-history only does assimilation rate reach an intensity comparable with that in the fully manured series. Over the rest of the range the remarkably low assimilatory capacity is reflected in what is a subnormal respiration rate for this series (it should be remembered that the respiration rates were observed *after* a period of assimilation, so that carbohydrate concentration during the respiration period must have been highly correlated with the assimilation rates observed). It follows, then, that only in the first few and the last leaves in this series is there any measure of the intensity of respiration under conditions of adequate supply of carbohydrate. It is highly significant that these particular leaves from Series D have *higher* respiration rates than those from the other three series, when the rates are expressed in terms of dry weight, the only really comparable basis. In fact, the two first determinations on the last leaf, where, judging from the assimilation rate curves, the internal concentrations of carbohydrate must be comparable in all the series, increasing potash starvation is reflected in a continually rising respiration rate from A to D (see Fig. 3, curves 10 and 11). The rise from A to B is great, and that from B through C to D more gradual; since the internal concentration of potash within the leaves shows a sharp drop from A to B, and a further small decrease from B to D, there is some indication that so long as carbohydrate content is not in the minimum, the rate of respiration between treatments (as distinct from successive leaves from the same treatment) is negatively correlated over the whole range with potash concentration.

These considerations indicate that the very high rate observed in Series D at the third leaf is indeed a real one, and that there is a high interaction between treatment and time due largely to the different behaviour of this particular leaf; there is thus every justification for omitting these four values from the Analysis on p. 374.

The immediate cause of this potential continual increase of respiration rate with increasing potash starvation remains obscure. Under conditions of low potassium supply and ample nitrogen a high percentage of nitrogen in the plant may be anticipated, leading possibly to a larger percentage of protoplasm in the potassium deficient leaves than in the leaves of fully manured plants. Work now in progress in this Institute has shown that higher percentages of nitrogen under conditions of potassium deficiency may be obtained. Furthermore, Morse (9) apparently found increases in the percentage of total nitrogen of the vegetative parts of soy beans as a result of potassium deficiency. Nightingale, Schermerhorn, and Robbins (10) also report slight increases in tomato. Whether from these observations, however, it may be concluded that the percentage of protoplasm has been increased seems doubtful. The latter workers rightly discriminate between storage proteins and active proteins of the protoplasm.

They further comment on the high amide and amino nitrogen content of the potassium deficient plants as compared with the fully manured controls. Similar results were found by Burrell (2) in soy beans, and have been confirmed for barley by the author in work now in progress. In this connexion the observations of Spoehr and McGee (11) on the accelerating effect of amino acids on the respiration rate of leaves is highly suggestive; and as a provisional hypothesis the increased respiration of leaves of potassium deficient plants may be attributed to the increase in amino acid content.

One further suggestion may be made in relation to the respiratory effect. Evidence has accumulated showing that potash concentration has a great influence on the production of at least some enzymes. Thus Doby (3, 4) found that in sugar beet, potato, rye, &c., amylase and saccharase are present in much greater quantities in leaves from potash-starved plants than in those from completely nourished ones. Whether 'respiratory enzymes' also are affected is uncertain, and the point need not be laboured at present, though the possibility is worth bearing in mind.

The water content data presented, and those previously published (5) indicate that under the conditions described leaves of barley are more succulent under potassium deficiency than in its presence. Our knowledge of the relationship between succulence and potassium supply is in an uncertain position, and some workers have apparently obtained results diametrically opposite to those here presented. Thus, Janssen and Bartholomew (6, 7), working with a variety of plants in sand, water, and plot culture, obtained consistently reduced water contents under reduced potassium supply, and state that their data 'show quite conclusively that high potassium plants are more succulent than low potassium plants'. The divergence between the two sets of results can scarcely be due to the differences in the plants used, since they included oats and Sudan grass; and the author has obtained increased succulence under reduced potassium, not only with barley, but also consistently with leaves and stems of three species of grasses, i.e. Italian rye-grass, cocksfoot, and rough-stalked meadow grass. The nutrient solutions used were, however, very different; those of Janssen and Bartholomew were entirely free from sodium, and the potassium was applied as chloride; moreover, in their case the concentration of salts at the roots was maintained approximately constant throughout the period of their experiments. It may be mentioned that Nightingale, Schermerhorn, and Robbins (10) working with tomatoes in a very similar manner to Janssen and Bartholomew, except that potassium was replaced by sodium where the former was omitted, obtained decreased succulence under potassium deficiency in the early stages of growth, but increased succulence in the later growth.

Janssen and Bartholomew (6), and Nightingale and his co-workers (10) also report that the leaves of tomato are darker green under potassium deficiency; this again is very different from the case of the barley of the present experiments, in which, as mentioned previously (5), deficiency is characterized by light yellow-green leaves.

SUMMARY.

1. The part played by water content in determining the differences in the usual characteristics between leaves from barley grown under various types of mineral salt deficiency is discussed; the conclusion is reached that differences in the ratio of dry weight to leaf area between treatments are almost wholly accounted for by differences in water content, whereas the variation of this ratio from leaf to leaf on the same plant is due primarily to variation in anatomical structure.

2. Results of experiments on the respiration rate of the successive leaves from plants grown at four external potash concentrations are presented. They show clearly that, in general, as the level of potash concentration is lowered, respiration rate increases, but that there is an optimum concentration below which the rate again decreases.

3. The positive correlation between respiration rate and amount of potash supplied, at very low concentrations, is apparently entirely due to the fact that carbohydrate concentration within the leaf is in the minimum. When abundant carbohydrate is present, the evidence is that over the complete range of manuring used there is a negative correlation between respiration rate and amount of potash supplied. A theory based on the amino acid content of the leaf is put forward in explanation of this.

4. As the external concentration of potash decreases so does the internal, but the relationship is not linear. There is strong evidence that where the amount of potash within the leaves is high, much of it may be washed out by rain, but under conditions of starvation what potash there is present cannot be removed in a like manner.

The author wishes to express his thanks to Sir E. J. Russell, through whose kindness this work was performed at Rothamsted; and to Professor V. H. Blackman, and Dr. F. G. Gregory, for their continued interest and ready assistance in the problems encountered.

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XXVI. STUDIES ON THE GUMS.

II. TRAGACANTHIN—THE SOLUBLE CONSTITUENT OF GUM TRAGACANTH.

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(*Received December 1st, 1931.*)

The literature on the subject of gum tragacanth, like that of the gums in general, is fragmentary and confused. The first work of value is that of Ogle [1900] who stated that this gum is not a simple substance, but contains 36 % of a water-soluble carbohydrate constituent and 42 % of an insoluble one, which swells up in water. The soluble constituent was stated to give a product on precipitation with alcohol, different from arabin (acid gum arabic). Widstoe and Tollens [1900] treated gum tragacanth as a simple substance, from which they obtained on hydrolysis the sugars, fucose and arabinose, and from another sample, xylose and arabinose. In the same year Hilger and Dreyfus [1900] stated that this gum contained 15–22 % galactose, estimated as mucic acid, and 30–42 % arabinose. Tollens [1901], as a result of ultimate analyses, formed the opinion that this gum contained carboxyl groups, a view contrary to that held by Hilger and Dreyfus. O'Sullivan [1901] gave an account of some very detailed work on the composition of this gum. He opens with the statement that gum tragacanth had been held to be a mixture of "cellulose, starch, bassorin, a gum-like arabin, a little nitrogenous matter, sugar, and ash." The soluble constituent "gum-like arabin" he concludes to be a mixture of polyarabinon-trigalactan-geddic acids and gives for it the formula

$$x\,[C_{10}H_{16}O_8 . 3C_{12}H_{20}O_{10} . C_{23}H_{38}O_{22}].$$

More recently von Fellenberg [1914] states that whereas the bassorin or insoluble portion is methoxylated to the extent of 5·38 %, the water-soluble constituent has no ester group of this type.

It seems, strangely enough, that a name has never been given to this constituent of gum tragacanth soluble in water, and it is proposed to term it tragacanthin.

Experimental.

Gum tragacanth on addition of water swells enormously, since the water-insoluble form, bassorin, constituting some 60–70 % of the gum, gives a very bulky jelly. O'Sullivan [1901] stated that he effected a separation of the soluble gum from the bassorin by means of fractional precipitation with alcohol. A solution of gum tragacanth in water was concentrated until a gelatinous scum began to form on the surface of the liquid. Dilute alcohol was added gradually until a bulky and curdy precipitate came down, which was stated to be bassorin. From the alcoholic filtrate, on evaporation and addition of strong alcohol, a further precipitate was obtained, claimed to be the tragacanthin. This he fractionated several times, but nevertheless it cannot be held to be an ideal method of separation. Some more reliable method was sought for in this work, and many methods both physical and chemical were tried. For a time a stream-line filter works admirably with a suitable grade of paper, but unless it is very large it chokes before any considerable quantity has filtered. Finally, filtration on very large fluted filter-papers was adopted. This was an extremely laborious method since filtration was slow, and since a concentration of the gum higher than about 0·1 % could not be employed. The filter-papers have frequently to be changed; but nevertheless such a method makes it absolutely certain that the product obtained is tragacanthin only. The very dilute filtrate was concentrated under reduced pressure to a small bulk, and several volumes of 95 % alcohol were added, together with a small quantity of hydrochloric acid. A stringy precipitate formed, not unlike many pectin preparations in appearance. This was filtered off, dissolved in water, and again precipitated with acid alcohol. After several such reprecipitations the product was dried in absolute alcohol and finally in a desiccator over phosphorus pentoxide.

A satisfactory separation was also effected in the following manner. The solution of the gum was first made alkaline and then just slightly acid by the addition of acetic acid, whereupon the physical condition of the bassorin is somewhat altered, and it appears as large gelatinous flocks. These were removed by running the liquid through a super-centrifuge. The effluent from the centrifuge was allowed to stand and the clear supernatant liquid finally filtered through filter-paper as before. The final product in each case was a fine white powder very readily soluble in cold water.

A portion was hydrolysed by boiling for one hour with sulphuric acid, and the solution examined for sugars after the removal of the acid by baryta. Three volumes of hot alcohol were added to the hydrolysate to remove incompletely hydrolysed gum and the filtrate concentrated under reduced pressure to a thin syrup which was then utilised for the tests. Contrary to the observation of O'Sullivan [1901] galactose was not found, for only a trace of mucic acid remained on oxidation with nitric acid. The only sugar that could be detected was arabinose, which was present in some quantity. This was identified by means of its diphenylhydrazone.

The yield of carbon dioxide as a measure of uronic acid content was determined in the usual way.

Tragacanthin. Sample I	Ash	0·87 %
Yield of CO_2 on ash-free basis	(i)	12·65 %
	(ii)	12·75 %

Mean value 12·70 % corresponding to 50·8 % uronic acid anhydride.

It is not possible to be definite as to the nature of the uronic acid, though there is some evidence for supposing that it is galacturonic.

The yield of furfuraldehyde was next determined so that a figure could be obtained by calculation for the pentose present in the molecule.

Yield of furfuraldehyde on ash-free basis... ...	(i)	31·26 %
	(ii)	31·42 %
Mean value		31·34 %

Since uronic acids themselves yield 16·66 % of furfuraldehyde, the 50·8 % uronic acid anhydride present in the molecule is responsible for the yield of 8·48 % furfuraldehyde, leaving the balance 22·88 % as due to pentose. This, calculated as anhydroarabinose, is 43·12 %.

Since no sugar other than arabinose could be detected in the hydrolysis liquid, it seems likely that tragacanthin consists solely of uronic acid and arabinose, though it is only possible to account for 94 % of the molecule in this way. There is a great divergence between this conclusion and that of O'Sullivan [1901], who considered tragacanthin to be a complex poly-arabinon-trigalactan-geddic acid, yielding on hydrolysis, arabinose, galactose, and geddic acid, an isomer of arabic acid also obtained by him from certain constituents of gedda gum. It seems likely that the preparation obtained by O'Sullivan contained some bassorin, which would account for the presence of galactan units.

To throw further light on the constitution of this gum several hydrolyses were carried out, and an examination of the hydrolysis products was undertaken. A portion of tragacanthin was dissolved in 3 % sulphuric acid and boiled under reflux for the period stated. It was then nearly neutralised with barium carbonate while still hot, filtered and poured into hot alcohol. A precipitate rapidly settled out and the supernatant liquid was poured off before it cooled. The precipitate was redissolved, filtered and reprecipitated with hot alcohol as before. This process was repeated several times in each case to ensure the removal of all free sugar. Finally it was dried with absolute alcohol and over phosphorus pentoxide.

The following analytical figures were obtained for the various preparations:

Table I. *Hydrolysis of tragacanthin with 3 °/₀ sulphuric acid.*

Preparation	Tragacanthin sample I %	30 minutes' hydrolysis %	60 minutes' hydrolysis %	90 minutes' hydrolysis %
Ash	0·87	1·04	2·87	3·70
CO_2 yield	12·70	18·83	19·27	20·28
Furfuraldehyde yield	31·34	23·76	23·18	22·20
Uronic acid anhydride	50·80	75·32	77·08	81·12
Furfuraldehyde from uronic acid	8·46	12·55	12·84	13·50
Furfuraldehyde from pentose	22·88	11·21	10·34	8·70
Anhydroarabinose	43·10	20·89	19·27	16·25

The composition of the hydrolysis products is illustrated in Fig. 1. It will be noted that during the initial period of hydrolysis the arabinose content diminishes rapidly, and the uronic acid increases proportionately. After 30 minutes, however, very little more arabinose is removed during a further period of 30 minutes, and even up to 90 minutes the product remains of much the same order. After this period hydrolysis soon becomes complete. These results throw a considerable amount of light on the constitution of the tragacanthin molecule. It is clear that the uronic acid and a portion of the arabinose form a more resistant nucleus acid, probably linked in the form of a ring. Attached to this by a glucosidic linkage must be the remaining arabinose, which is thus easily removed on hydrolysis. A simple arrangement agreeing fairly closely with the figures experimentally obtained is a nucleus acid consisting of three molecules of uronic acid linked with one of arabinose to form a four-membered ring, attached to which is a side chain of two molecules of arabinose, as illustrated diagrammatically below.

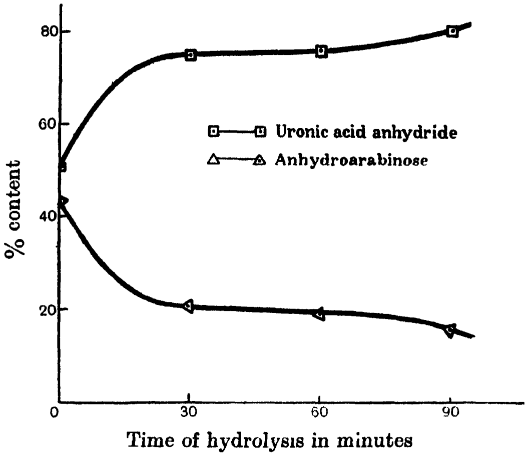
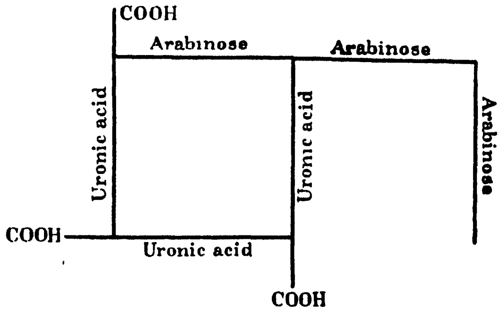


Fig. 1. Hydrolysis of tragacanthin with 3 % H_2SO_4.



Such a nucleus would have a composition of approximately 20·0 % anhydroarabinose and 80·0 % uronic acid anhydride, which is not dissimilar from that corresponding with the figures obtained after hydrolysis for a period of 1 hour. It is, of course, not claimed that the constitution of this gum is represented above, but the evidence obtained from a study of the course of hydrolysis strongly suggests this general type of arrangement. A study of gum arabic [Norman, 1929] on similar lines led to the conclusion that it also contains a nucleus acid, resistant to mild hydrolysis, to which are attached arabinose units by a type of linkage easily broken. A similar type of arrangement has just been described for mesquite gum by Anderson and Otis [1930]. In this substance they find a resistant nucleus containing three units of galactose and one of methoxyglycuronic acid, to which are attached through a dicarbonyl linkage, four units of arabinose. It is probable therefore that this general arrangement may be found to hold for other members of this group of substances. Bassorin, which is very much more complex in structure than tragacanthin seems to be similarly constituted.

SUMMARY.

1. Tragacanthin, the soluble constituent in gum tragacanth, may be separated by ordinary filtration in extreme dilution.

2. Uronic acid units are found to be present and to constitute about one-half of the molecule. Arabinose was the only sugar found; no galactose could be detected.

3. Hydrolysis products were prepared, the analytical figures for which give rise to the suggestion that a portion of the arabinose is united to the uronic acid to form a resistant nucleus, and the residue attached by glucosidic linkage, and therefore easily removable.

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CCXIII. THE PRODUCTION OF MUCUS DURING THE DECOMPOSITION OF PLANT MATERIALS[1].

I. THE EFFECT OF ENVIRONMENTAL CONDITIONS.

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It has been observed that the straw in some manure heaps undergoing decomposition develops stickiness. Such sticky manures may be better adapted to light sandy soils and are possibly unsuitable for heavy clays. The possible effect of mucilage from plant residues on the physical behaviour of the soil has been suggested by Hutchinson and Clayton [1919]. The substance conferring on manures the physical property of stickiness will be referred to hereafter as mucus. It is apparent that mucus formation must be a result of the microbiological activity during decomposition.

Though the decomposition of plant materials has been studied extensively and the losses of individual constituents followed in detail by Rege [1927], Waksman [1928] and Norman [1929], no reference is made in the literature to the production of mucus.

The experiments described in this paper were designed to examine the conditions involved in the production of mucus. Straw was fermented in the presence of different sources of available nitrogen with changes in the physical conditions and reaction. Extractions of the decomposed straws were made to seek any possible correlation between the rates of their decomposition and the production of mucus. The amount of mucus produced was measured by a specially devised physical test.

Physical test for measuring the stickiness.

The principle of the method consists in measuring the vertical force required to separate after drying two metal plates which contain between them a known weight of the manure sample.

The following apparatus is required for the determination—a number of pairs of metal plates, one of 4 ins. square and the other of 3 ins. square with a hook in the centre, a system of two frictionless pulleys in a horizontal plane, lead shot, a receptacle to receive the lead shot and a lead weight weighing 1600 g. with a slit to fit over the hook of this plate. This apparatus is represented diagrammatically in Fig. 1.

The metal plates should be sufficiently rigid not to bend under the forces applied. In order to keep the lower plate firmly fixed, while the force is being applied, it is made to slide in between two jaws made by screwing to the bench top two metal pieces over another of 4 ins. length and of the same thickness as that of the plates. The lower two plates are kept parallel and 4 ins. apart in such a way that the lower metal plates of 4 ins. square can be easily slid in with no danger of causing any jerks to the plates containing the manure. When the plates are ready for measuring the pull, the lower plate is gently slid in, the loop at one end of the string over the pulleys is put in the hook of the top plate and the other is connected with the receptacle for carrying the lead shots. The shots are gradually poured in till the top plate is vertically lifted instantaneously from its position of rest. The weight of the shots *minus* the weight of the top plate gives the total pull to separate the two plates. The diameter of the circular block occupied by the manure is measured. An average of three or four readings in different positions is taken as the block is not usually regular. Knowing the diameter and the pull recorded, the force per unit area can be calculated.

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[1] This paper is an abridged form of a part of the thesis approved for the Degree of Doctor of Philosophy in the University of London.

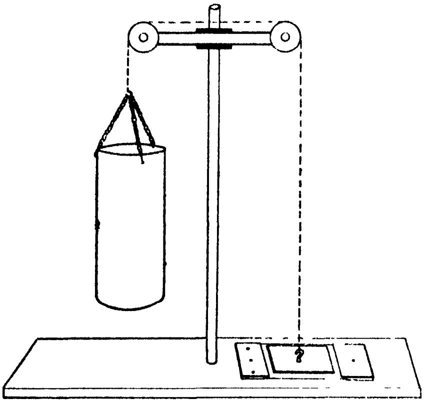

Fig. 1. Apparatus used for measuring stickiness.

The procedure consists in cutting the rotted straw as finely as possible, after which the mass is thoroughly mixed and vigorously worked with a spatula to obtain an even mixture. One gram portions are then weighed out on each of the plates. The mass is gathered into a mound by the spatula in the centre of the plate and the top plate placed in such a way that it rests freely with faces parallel. It is then pressed with the lead weight for 15 seconds and dried at 100° in an oven overnight to ensure uniform and complete drying. It is essential in all the experiments to keep constant the weight and time for pressing the plates, because the area of the manure and its height or thickness depend upon the force with which the top plate is pressed against the manure surface, whilst the pull required to separate the two plates depends upon the distance between them and therefore upon the thickness of the manure disc. In his early experiments on the stickiness of soils Kachinski [1930] left a weight on the sample for a specified time. Bouyoucos [1932] however pressed the plates with the hands, introducing different pressures at different times, without considering the thickness of the disc occupied by the soil.

Table I gives the significance of the determinations of stickiness made by the physical test. It will be seen that the magnitude of the error increases inversely with stickiness and that with highly sticky and moderately sticky samples the figures for stickiness are significant.

Table I. *Significance of the determinations of stickiness made by the physical test.*

Readings	Actual pull recorded in g.	Radius of the manure disc in cm.	Force per unit area per 1 g. of wet sample	Force per unit area per 1 g. of dry sample
$NaNO_3$ rot: dry matter 13 %. Very sticky.				
1	5200	1·25	1059	8140
2	4875	1·25	991	7620
3	4958	1·25	1010	7770
4	4800	1·20	1061	8160
5	4110	1·25	837	6436
6	5190	1·25	1056	8120
7	5000	1·25	1018	7830
	5075	1·25	1034	7944
		Mean		7752·5
		Standard deviation		566·32
		Standard error of the mean		± 200·2
Urea rot: dry matter 20 %. Moderately sticky.				
1	2000	1·30	481·4	2407
2	1800	1·25	366·4	1832
3	1200	1·25	244·5	1222
4	1600	1·20	353·7	1768
5	1900	1·20	420·1	2100
6	2000	1·25	407·3	2036
7	2050	1·25	417·4	2087
8	1800	1·20	390·0	1990
		Mean		1930·2
		Standard deviation		345·0
		Standard error of the mean		± 121·9
$(NH_4)_2CO_3$ rot: dry matter 12 %. Slightly sticky.				
1	130	1·80	12·7	105·8
2	150	1·75	13·5	101·2
3	100	1·40	16·2	135·3
4	150	1·70	16·5	137·7
5	290	1·60	36·0	300·5
6	100	1·60	12·4	103·5
7	250	1·50	35·3	294·1
8	220	1·60	27·3	227·5
		Mean		175·7
		Standard deviation		85·4
		Standard error of the mean		±30·2

EXPERIMENTAL.

The physical test for stickiness was carried out on decomposed straws obtained under the various conditions outlined below.

Oat straw was rotted in presence of mixed flora for 30 days with the following changes in the environmental conditions.

(*a*) Variation in the initial moisture content at 35°.

(*b*) Variation in the sources of nitrogen at 35°.

(*c*) Variation in temperature—15°, 25°, 35° and 45°—with urea as the source of nitrogen.

(*d*) Variation in temperature—15°, 25°, 35° and 45°—with sodium nitrate as the source of nitrogen.

(*e*) Degree of decomposition at 35° with sodium nitrate as the source of nitrogen.

(*f*) Adjustment of p_H at 10·0 independent of the source of nitrogen.

Technique and methods.

Twenty g. of air-dry chaffed oat straw of known moisture and nitrogen contents were fermented aerobically in bottles with its natural mixed flora. Nitrogen was supplied to the extent of 1 g. per 100 g. of straw and the bottles incubated at the desired temperature. They were turned round in the first few days to ensure thorough wetting and frequently stirred to get homogeneous distribution of nitrogen and moisture. Water-logging was avoided as it causes anaerobic conditions. After the desired period each bottle was weighed with its contents and analysed as indicated below.

1. Extractions with water, 1 % sodium carbonate and 1 % sodium hydroxide. In each case 10 g. of the wet sample were boiled for 5 minutes with 100 cc. of water and filtered. The aqueous extract was evaporated to dryness on a water-bath and weighed. The alkaline extracts were precipitated with a few drops of hydrochloric acid, gently heated to coagulate the precipitate and then filtered through tared papers. The precipitates were well washed, dried and weighed.
2. Extractions with 90 % alcohol were carried out in a Soxhlet apparatus for 6 hours. Upon removal of the alcohol the extract was weighed, and after hydrolysis by boiling with 5 % H_2SO_4 for 2 hours its sugar content was determined by the Mohr-Bertrand method.
3. Treatment with hydrogen peroxide: the method employed was described by Shrikhande [1933].
4. Ammonia-N was determined by distillation with MgO.
5. Nitrate-N on the samples rotted with sodium nitrate was determined by distillation of the residue from ammonia-N in presence of alkali and Devarda's alloy.
6. Total N was determined by the usual Kjeldahl method. In the presence of nitrate-N the sulphuric-salicylic acid method was adopted.

RESULTS.

Series (*a*) *and* (*b*). Fungi were noticed on or about the 6th day. At the end of 30 days no trace of fungus mycelium was obvious except with ammonium carbonate and urea. *Coprinus* seemed to be very active in ammonium carbonate, urea and sodium nitrate rots. Better decomposition was observed with ammonium sulphate when calcium carbonate was introduced. Fungus growth was hardly noticeable at 15°. The rots appeared different under different conditions. The manures obtained, using ammonium carbonate, sodium nitrate and mould tissues as the source of nitrogen at high moisture contents, were noticeably slimy.

Effect of initial moisture content.

Table II gives the effect of initial moisture content upon the decomposition and nitrogen transformation. In each case the moisture content initially was adjusted at 60, 70, 80 and 90 %. During the course of decomposition an attempt was made to maintain the above levels of moisture but at the end of 30 days they seem to narrow down to about 85 to 98 %, which seems to be the optimum moisture necessary for pronounced rotting.

The series with ammonium carbonate, ammonium sulphate and sodium nitrate were repeated twice. Series (*a*) was used for nitrogen determinations and series (*b*) for extractions and the physical test for stickiness. The figures for moisture content and the losses of dry matter indicate that the higher the moisture content the greater the activity of the organisms and consequently the greater is the degree of decomposition. This observation agrees with that of Engberding [1909] who found an increased number of bacteria with the increased

Table II. *Nitrogen content of straws rotted by mixed floras with different sources of available nitrogen for 30 days at 35°.*

Expressed on 100 g. dry straw.

Initial moisture %	Dry matter	Loss of D.M.	NH_3-N	NO_3-N	Total N	N factor	N equiv.
			Ammonium carbonate.				
60	(*a*) 13·8	39·6	0·018	0·0	1·20	0·89	2·2
	(*b*) 19·7	36 5					
70	(*a*) 12·8	40·6	0·016	—	1·33	0·96	2·3
	(*b*) 15·0	39·2					
80	(*a*) 12·0	41·2	0·013	—	1·33	1·06	2·5
	(*b*) 14·0	45·0					
90	(*a*) 11·9	42·2	0·044	—	1·71	1·20	2·8
	(*b*) 11·4	44 4					
			Ammonium sulphate.				
60	(*a*) 17·0	36·5	0·467	—	1·29	0·68	1·8
	(*b*) 23·4	30·1					
70	(*a*) 15·9	28·5	0·595	—	1·49	0·59	2·1
	(*b*) 19·3	32·5					
80	(*a*) 15 4	23 4	0·550	—	1·41	0·69	3·0
	(*b*) 16·7	31·5					
90	(*a*) 14·0	20 0	0·593	—	1·68	0·68	3·4
	(*b*) 14·7	30·7					
			Sodium nitrate.				
60	13·8	30 7	0·041	0·119	1·76	0·83	2·7
70	(*a*) 13·6	49·2	0·012	0·020	1·18	0·81	1·6
	(*b*) 15·2	52·8					
80	(*a*) 13·4	43·7	0·009	0·009	1·20	0·63	1·4
	(*b*) 13·3	52·3					
90	12·6	48·7	0·027	0·027	1·31	0·89	1·8
			Water.				
80	18·2	30·0	—	—	0·36	0·16	5·3
			Peptone.				
75	16·5	39·5	0·004	—	1·48	1·12	2·8
75	15·6	44·1	0·007	—	1·49	1·10	2·4
			Caseinogen.				
75	17·0	43·0	0·002	—	1·46	1·10	2·5
75	17·1	41·8	0·002	—	1·30	1·07	2·5
			Urea.				
75	20·0	32·5	0·003	—	1·36	1·03	3·1
75	18·9	34·0	0·004	—	1·67	1·30	3·8

moisture content of the soil. There is, however, an exception to this in the case of ammonium sulphate where the order is reversed. It is possible therefore that a high moisture content with $(NH_4)_2SO_4$ as the source of nitrogen depresses the activity of the organisms.

The figures for "nitrogen factor" of straws rotted at 35° were determined for the sake of comparison and are given in column 7 of Table II. The nitrogen factor in these cases is the resultant of the many organisms involved and shows a tendency to increase with higher moisture content, meaning that it increases with the degree of decomposition for the particular source of nitrogen. Ammonium sulphate gives the lowest nitrogen factor whereas ammonium carbonate gives the highest with inorganic sources of nitrogen. Sodium nitrate however does not appear so good as ammonium carbonate from the point of view of nitrogen immobilisation although it gives a higher decomposition. The nitrogen factor with organic sources of nitrogen is of little value because it is difficult to distinguish between nitrogen synthesised by the organisms and the organic nitrogen supplied.

"Nitrogen equivalents" are given in Table II, column 8. Sodium nitrate gives the lowest figure of 1·4 with 80 % initial moisture content indicating the greatest activity of the organisms per g. of N. The highest figure is 3·4 with $(NH_4)_2SO_4$ and 80 % moisture, no doubt due to the low activity of the organisms in this case.

Hutchinson and Clayton [1919], while discussing the decomposition of cellulose with *Spirochaete cytophaga*, say "from the chemical standpoint and on account of its insolubility in acids and solubility in ammonium hydroxide, the mucilage would without doubt appear in the 'crude humus' fraction in the conventional soil analysis." An attempt was made to extract this fraction possibly responsible for the stickiness. Table III gives the figures for various extracts and the values for the stickiness as measured by the physical test. The figures for stickiness were obtained on the mixture of samples with the different moisture contents for a particular source of nitrogen. The maximum stickiness was obtained with sodium nitrate, which also gave the greatest decomposition and the highest water, sodium carbonate and sodium hydroxide extracts. Mould tissue is the source of nitrogen which gives the maximum decomposition. 2 % nitrogen supplied as tissue gives extracts of the same order as sodium nitrate though the stickiness is rather lower. Double the amount of tissue produces more than double the amount of stickiness. This clearly indicates that stickiness depends a great deal upon the quantity of elaborated fungal tissue. The lowest figures for stickiness and extracts were obtained with ammonium sulphate. The best correlation between stickiness and extracts seems to be obtained with sodium carbonate extraction. This is expressed graphically in Fig. 2. The logarithm of stickiness when plotted against the extract can approximately be represented by a straight line. Log $y = 0{\cdot}0983x + 2{\cdot}365$.

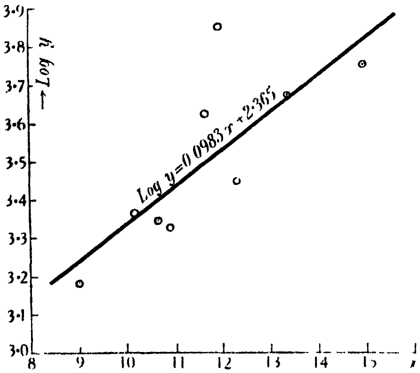

Fig. 2. Correlation between stickiness and sodium carbonate extracts of manures.

The aqueous extract increases with the degree of decomposition. Water would extract some protein, very little of the hemicelluloses and any water-soluble material, gummy or otherwise, synthesised during decomposition. The larger water extract can therefore be explained by the presence of more of the

Table III. *Different extracts with physical test for stickiness and* p_H *values of straws rotted by mixed flora with different sources of nitrogen for 30 days at 35°.*

Expressed on 100 g. dry manure.

Initial moisture %	Loss of D.M.	Alcoholic extract	Sugars in alcoholic extract mg.	H_2O extract	NaOH extract	Na_2CO_3 extract	Physical test g.	Initial p_H	Final p_H	H_2O_2 extract
					$(NH_1)_2CO_3$.					
60	(*a*) 37·16	5·16	1139·5	—	—	—	4241	9·0	8 0	20·2
	(*b*) 36·50	6·20	—	21·1	16·9	11·5				
70	(*a*) 40·60	5·04	326·9	—	—	—				
	(*b*) 39 20	8·50	—	21·2	18·2	11·9				
80	(*a*) 41 20	4·30	462 5	—	—	—				
	(*b*) 45·00	5·20	—	22 2	11·4	8·8				
90	(*a*) 42 20	4 58	382·2	—	—	—				
	(*b*) 44 40	10 30	—	29·3	13·5	3·5				
					$(NH_4)_2SO_4$.					
60	(*a*) 36 50	4 19	418 0	—	—	—	220	5·5	6·5	14·5
	(*b*) 30 10	9·00	—	17·9	16·8	7·7				
70	(*a*) 38 50	4·45	529·0	—	—	—				
	(*b*) 32·50	8·20	—	20 5	13·3	4·1				
80	(*a*) 23·40	4·50	520 0	—	—	—				
	(*b*) 31·50	9·20	—	20 8	14·4	3·3				
90	(*a*) 20 0	5 30	513·0	—	—	—				
	(*b*) 30 7	9 30	—	19·8	12 2	4·5				
					$NaNO_3$.					
60	30 7	5 50	247·6	—	—	—	7164	6·35	10·0	27·4
70	(*a*) 49 2	3 80	104·8	—	—	—				
	(*b*) 52·8	9 10	—	44 3	17 4	15 5				
80	(*a*) 43 7	4·30	109 8	—	—	—				
	(*b*) 52·3	10 30	—	62·1	19·3	8·3				
90	48·7	4 35	182·0	—	—	—				
					Water.					
80	30·0	6 35	—	12·9	10 5	1·2	0	5·50	—	16·3
					Peptone.					
75	39·5	8·25	—	21 5	15·2	10·9	1504	5·75	7·5	16·3
75	44·1	7·11	—	26·9	16·6	7·2				
					Caseinogen.					
75	43 0	7·10	—	28 7	24·5	11 9	2727	—	7·5	15·8
75	41·8	11·05	—	27·2	19·4	13·5				
					Urea.					
75	32·5	8·36	—	25 3	19 7	11·6	2278	6·45	7·5	12·8
75	34·0	10·04	—	19·0	8 7	8·7				
					$(NH_4)_2SO_4+CaCO_3$.					
80	35·9	—	—	—	—	10·6	2176	—	7·5	—
					$Ca(CN)_2$.					
80	29·1	—	—	—	—	13 2	4635	—	—	—
					Mould tissue to give 1 % nitrogen.					
80	57·7	—	—	28·4	10·3	17·9	2124	—	8·2	—
					Mould tissue to give 2 % nitrogen.					
80	61·6	—	—	36·9	10·0	20·3	5622	—	8·2	—

protein and other microbial constituents which are progressively synthesised and possibly also of more gummy material which seems to increase, as indicated by the high stickiness with the physical test.

The alkaline extracts diminish with the time of decomposition. Alkaline treatment extracts more than water alone, but only a part of the substances in solution is precipitated by acid. The materials extracted with alkali are practically of a similar nature to those obtained with hot water. Extraction may vary with increasing concentration and nature of the alkali. 1 % sodium hydroxide would extract lignin to an appreciable extent, and on comparing the figures for sodium hydroxide and sodium carbonate extracts it is seen that the sodium hydroxide extract is nearly one and a half times the sodium carbonate extract. The weight of the extracts increases as the stickiness increases.

Of organic solvents only alcohol was found to extract a certain fraction of manure and the humus from it. Alcoholic extracts are practically of the same order in each case. There is a tendency for the extract to increase with the degree of decomposition. The sugar content or the extract from ammonium carbonate on an average is greater than that from ammonium sulphate or sodium nitrate.

The significance of extraction with hydrogen peroxide is discussed in detail elsewhere [Shrikhande, 1933]. Much importance cannot be attached to the relationship between peroxide extract and stickiness. The nature of this extraction differs from others in the sense that this solvent oxidises some of the decomposed and synthesised material. All extracts if plotted graphically against stickiness give a straight line. In the rots with caseinogen there is an exception to the general order as mentioned above, all the extracts being disproportionately greater. This may be due to the fact that less caseinogen is decomposed by the organisms, and the remainder is dissolved by the solvents and returned unchanged in precipitates.

The initial p_H was highest with ammonium carbonate and lowest with ammonium sulphate. Sodium nitrate which was practically neutral at the start gave a final p_H of 10·0. The highest stickiness and decomposition were obtained with sodium nitrate and correspondingly lowest figures with ammonium sulphate, which maintained an acid reaction throughout. When, however, an equivalent amount of calcium carbonate was supplied to the ammonium sulphate rot, decomposition increased with an increase in stickiness. This leads to the conclusion that maximum stickiness is associated with alkaline conditions. This may mean either that the organisms responsible for stickiness are favoured by an alkaline medium, that there is a modification of the flora with the change in the environmental conditions or that the manifestation of the property of stickiness is enhanced by alkaline conditions. The p_H with all the three organic sources of nitrogen is practically of the same order (7·5) and similar figures for stickiness were obtained. The final high p_H with sodium nitrate is no doubt due to the utilisation of nitrate-N leaving excess of base. The slight lowering of p_H with ammonium carbonate may be ascribed to the use of nitrogen from ammonia and the liberation of CO_2.

Table IV. *Different extracts with physical test for stickiness and final* p_H *values of straws rotted by mixed floras with available nitrogen as urea and* $NaNO_3$ *at different temperatures.*

Expressed on 100 g. dry manure.

Temp. ° C.	Source of N	Loss of D.M.	Physical test (g.)	Final p_H	Water extract	Na_2CO_3 extract	NaOH extract
15	Urea	7·2	414	7·8	17·1	7·7	13·2
	$NaNO_3$	12·3	2610	9·0	23·2	5·2	10·5
25	Urea	40·6	5020	8·0	29·6	11·9	16·0
	$NaNO_3$	30·2	3678	9·5	31·8	8·5	17·5
35	Urea	33·0	2278	7·5	25·3	11·6	19·7
	$NaNO_3$	52·5	7164	10·0	44·3	15·5	19·3
45	Urea	58·2	3650	8·5	38·7	18·1	19·8
	$NaNO_3$	40·9	7875	10·0	47·5	18·4	22·3

Series (*c*) *and* (*d*). *Variation in temperature with urea and sodium nitrate as sources of nitrogen.* Table IV contains results of extraction, final p_H and stickiness on samples of straws obtained as indicated above.

The losses of dry matter seem to increase with the increase in temperature. At 15° the decomposition is very poor in both cases, though sodium nitrate gives double that of urea. The decomposition obtained with urea at 25° is more than that at 35°. At 45° urea gives a decomposition of 58 %, which is much more than with sodium nitrate. These variations clearly indicate the different nature of the flora working at the different temperatures. The final p_H for urea varies between 7·8 at 15° and 8·5 at 45°. Similarly the p_H with sodium nitrate has increased from 9·0 at 15° to 10·0 at 45°. The initial p_H values were 6·45 and 6·35 respectively.

Stickiness. Even with a very small decomposition sodium nitrate produces quite an appreciable amount of stickiness, which increases with the rise in temperature. The maximum stickiness with urea as a source of nitrogen is produced at 25°.

Series (*e*). *Degree of decomposition at 35° with sodium nitrate as the source of nitrogen.* Table V indicates the relationship observed between the stickiness of the manure and the degree of decomposition when sodium nitrate was supplied as the source of nitrogen. The physical test increases markedly as decomposition proceeds. There was, however, a possibility that this might be an effect of the changing reaction of the rot, which becomes progressively more alkaline and reaches finally a p_H of 9·5. To investigate this point the samples after 8 and 16 days' decomposition were adjusted to the final p_H of 9·5 by addition of sodium carbonate. This had the effect of increasing the figures for the physical test very appreciably, but not so much that they approached the level obtained at this p_H with longer periods of decomposition. There is therefore a direct relationship between the degree of decomposition of a manure and its stickiness, even when all the changes in the reaction have been taken into account. This point is more clearly brought out when the physical test is recalculated on a basis of original straw. The stickiness increased till the 24th day, after which further loss of organic matter was not accompanied by further production of apparent sticky material.

Table V. *Effect on the production of stickiness of modification of the* p_H *and the degree of decomposition.*

			Physical test in g.					
						p_H adjusted		
Source of N	Time in days	Loss of D.M. %	On manure	On original straw	Final p_H	To	With	On manure
$NaNO_3$	8	4·7	2050	1954	8·0	9·5	Na_2CO_3	3868
,,	16	23·1	4020	3903	9·0	9·5	Na_2CO_3	5521
						9·5	K_2CO_3	5123
						8·0	H_2SO_4	3648
						5·5	H_2SO_4	2305
,,	24	32·2	7834	5310	9·5	—	—	—
,,	32	34·8	8161	5320	9·5	8·0	H_2SO_4	5991
						7·0	H_2SO_4	4538
						5·5	H_2SO_4	3410

Further to demonstrate the effect of reaction, samples rotted for 32 days, having achieved the very high degree of stickiness indicated by a physical test of 8161 g., were acidified and the p_H reduced to 8·0, 7·0 and 5·5. This had the effect of lowering the stickiness to 5970 g., 4538 g. and 3410 g. respectively. Similar adjustments of the p_H with sodium carbonate, potassium carbonate and sulphuric acid were made with the sample rotted for 16 days. These experiments indicate that the stickiness varies directly with the p_H within the limits tested. The relation between stickiness and reaction is represented graphically in Fig. 3.

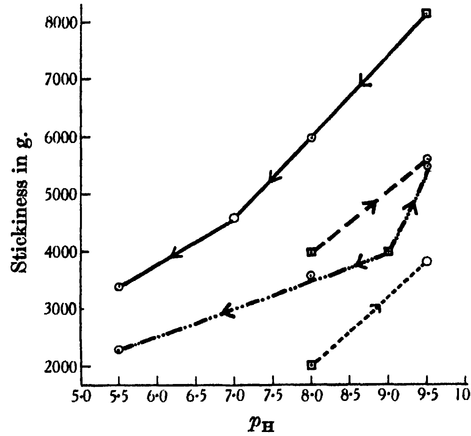

Fig. 3. Relationship between stickiness and reaction.

▣ Initial point. → Changes due to alteration in p_H.
——— $NaNO_3$ 30 days. — — Urea 30 days.
—··— $NaNO_3$ 16 days. - - - - $NaNO_3$ 8 days.

Series (*f*). *Adjustment of* p_H *at 10·0 independently of the source of nitrogen.* Table VI deals with the effect on stickiness of the initial reaction of the material undergoing decomposition. Various sources of nitrogen were supplied and the initial p_H of the straw adjusted to 10·0 with magnesium carbonate in one series and sodium carbonate in another. This particular reaction was chosen because the highest figure hitherto obtained for stickiness was found in a rot supplied with sodium nitrate which had attained finally that high degree of alkalinity. During fermentation the p_H values of both the series fell somewhat, those with magnesium carbonate more than those with sodium carbonate. The losses of dry matter and stickiness obtained were however invariably greater in the latter series. It is clear that if rots are adjusted initially to a high degree of

Table VI. *Effect of the initial* p_H *on the production of stickiness.*

Source of N	Initial p_H brought to 10·0 with	Final p_H	Loss of D.M. %	Physical test g.
Peptone	$MgCO_3$	8·0	35·3	3938
Peptone	Na_2CO_3	8·5	49·0	6490
Mould tissue as 1 % N	$MgCO_3$	8·0	37·0	4789
*Mould tissue as 1 % N	Na_2CO_3	8·0	52·2	4114
Mould tissue as 2 % N	$MgCO_3$	8·0	50·3	4863
Mould tissue as 2 % N	Na_2CO_3	8·5	56·5	5349
Urea	$CaCO_3$† (p_H: 9·0)	8·0	39·0	3991
Urea	Na_2CO_3	8·5	42·3	6001

* Water-logged. † Final p_H brought to 10·0 by adding Na_2CO_3. Stickiness then equals 5620 g.

alkalinity the manure resulting is stickier than that which is usually obtained. Furthermore it appears that, given the correct p_H, sodium or potassium ions are more favourable than are calcium and magnesium.

The effect of sticky manure on soils.

Three soils of different composition were selected, a Rothamsted soil which owing to a high clay content is very heavy, a Woburn and a Cheshire soil, both of which are light, but the former more sandy. Two grams of the soil sample were mixed as uniformly as possible with one gram of the sticky manure and made into a paste with water. The dry matter of the mixed sample was adjusted to about 50 %. The physical test was carried out on one gram of this mixture as in the case of manures. Stickiness of the soils when wet and after drying was also determined to compare with the figures after mixing them with the sticky manure. The following table contains these figures.

	Physical test in g.		
Soil	Wet	Dried	When mixed with manure and dried
Rothamsted	431	924	947
Cheshire	0	424	829
Woburn	0	214	686

There is therefore a definite increase in the stickiness of light soils on mixing with such a manure.

The nature of the mucus.

Attempts to extract the sticky constituents of the manure with different solvents give unsatisfactory results. The usual method of precipitating gums from water and mildly alkaline extracts with absolute alcohol and Fehling's solution was also tried without success.

A sodium nitrate rot with mixed flora, which was definitely sticky to the touch and markedly so by the physical test, was extracted with cold water. The extract was colloidal. It was filtered twice through glass wool and finally through a filter-paper under suction. The extract was then precipitated with a few drops of HCl. The precipitate was coagulated by gentle heating on a hot plate and then filtered on a Büchner funnel. A very small fraction of this mixed with 1 g. of dry oat straw proved to be quite sticky. On drying the precipitate became very hard and gritty and lost its binding properties in part. Having thus established that the above precipitate contains a sticky constituent both when wet and dry, the following analytical figures are obtained on the extract.

Water extract	9·86 % on dry matter
Physical test for stickiness with 1 g. of the wet manure	4880 g.
Physical test with the wet extract ...	2555 g.
Physical test with the extract dried and then re-moistened	1575 g.
On hydrolysis with 3 % H_2SO_4 for 5 hours	
Apparent anhydroglucose	52·04 %
Anhydropectose	2·46 %
Protein	21·50 %
Ash	3·20 %
Total	79·20 %

After acid hydrolysis the extract reduced Fehling's solution and gave Selivanoff's test for fructose. On oxidation of a portion with concentrated nitric acid a precipitate was obtained consisting of colourless plates and micro-sandy crystals. The crystals were separated by shaking up with hot alcohol in which the plates dissolved. The insoluble residue had M.P. over 200°, and when distilled with conc. HCl yielded furfuraldehyde indicating the presence of mucic acid.

The extract appears therefore to be a mixture of carbohydrates and proteins, and no doubt in part consists of material extracted from the elaborated microbial tissue. The carbohydrate portion of the extract seems to consist largely of galactan, though indications have been obtained also of the presence of uronic acids and a little pentose.

SUMMARY.

1. The conditions under which stickiness is produced in decomposing plant materials and manures have been investigated and some information obtained as to the nature of the substances contributing this property.

2. A physical test for evaluating the property of stickiness in manures has been described.

3. In the presence of a mixed natural flora, the chief factors involved in causing stickiness in decomposing straw are the source of nitrogen supplied, the initial and final reactions of the material and the degree of decomposition.

4. High values for stickiness are given with either sodium nitrate or mould tissues as the sources of nitrogen. This suggests that an alkaline reaction and an abundance of microbial tissue are essential in the production of stickiness during decomposition by mixed flora.

5. The final reaction of the manure profoundly influences the degree of stickiness, if at all appreciable. A p_H of 9·5 to 10·0, whether obtained by fermentation or by subsequent adjustment, seems to give the maximum stickiness. Sodium or potassium ions produce more stickiness than calcium or magnesium.

The author is indebted to Sir John Russell, Director of the Rothamsted Experimental Station, for placing at his disposal the facilities of the Station, and to Mr E. H. Richards, Head of the Fermentation Department, for suggesting the problem and for invaluable advice and criticism. Thanks are due to Dr A. G. Norman for his assistance and suggestions, and to Mr Scott Blair, of the Physics Department, for criticising the physical test.

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CCXIV. THE PRODUCTION OF MUCUS DURING THE DECOMPOSITION OF PLANT MATERIALS[1].

II. THE EFFECT OF CHANGES IN THE FLORA.

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THE production of stickiness during the process of decomposition of cellulosic materials has been attributed by Hutchinson and Clayton [1919] to the activity of soil organisms such as *Spirochaeta cytophaga*. Since the time of Mitscherlich [1850] bacteria were considered to be the chief agents in the natural decomposition of plant materials until Konig [1904] suggested the greater importance of fungi. McBeth and Scales [1913] and Scales [1915] found fungi to be capable of using cellulose as the sole source of energy. It has been shown by Waksman [1926] and later confirmed by Norman [1929] that in the presence of sufficient available nitrogen, cellulose is the chief constituent to be decomposed. Waksman [1924] and Rege [1927] have shown that fungi are mainly responsible for the decomposition especially during the early stages.

Norman [1931] has studied the nitrogen transformations and changes in the carbohydrate constituents with pure cultures of fungi but no such study has so far been made of the subsequent action of bacteria after fungal attack, or of the simultaneous action of a bacterium with that of a fungus. Apart from the study of stickiness produced during decomposition, it was thought worth while to follow the ammonification and nitrogen changes and the losses in carbohydrate constituents at different stages of decomposition.

The work described in this paper was carried out in order to test whether mucus is produced during decomposition of straw with pure cultures of organisms. Straw was therefore decomposed with selected fungi, fungi and bacteria and bacteria alone. Analyses of the different constituents were made in order to find any correlation between the rates of their decomposition and the formation of mucus. The amount of mucus produced was measured by the physical test described in Part I [1933].

Scheme of work.

(1) Oat straw was rotted with pure cultures of fungi at 35° with ammonium carbonate as source of nitrogen and analysed at intervals of 8, 16, 24 and 48 days.

(2) Straw was rotted with pure cultures of bacteria: (i) *Mycobacterium agreste*, (ii) *Spirochaeta cytophaga*, and analysed at intervals of 8, 16, 24 and 48 days to see if any bacterium which, like the latter, produces a gummy colony on an artificial medium would produce stickiness in straw.

(3) Straw was rotted first for 48 days with fungi alone: (*a*) and then inoculated with *M. agreste* and analysed on the 8th, 16th, 24th and 48th days after inoculation; (*b*) as (*a*) but with the substitution of *S. cytophaga* for *M. agreste*.

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[1] This paper is an abridged form of part of a thesis approved for the Degree of Doctor of Philosophy in the University of London.

(4) Straw was rotted with a fungus progressively and then inoculated with *S. cytophaga* on the 8th, 16th, 24th and 48th days, and analysed on the 8th and 40th days after inoculation with the latter.

(5) Straw was rotted simultaneously with fungus and *S. cytophaga* and analysed on the 8th, 16th, 24th and 48th days.

Methods.

Weighed quantities of straw were bottled and sterilised in an autoclave under 115 lbs. pressure for 45 minutes on two consecutive days. The treatment is undoubtedly drastic yet it is necessary for complete sterilisation. Available nitrogen was supplied as sterile ammonium carbonate in the proportion of 1 g. per 100 g. straw and the moisture was adjusted at about 80 %. A heavy inoculum of a suspension of the spores of the required organism in sterile water was then added to each bottle. Platings were made at the end of the experiments to test the purity of the organism. Fungal contamination was easily noticeable in most cases, since the straw was characteristically coloured by the spores of the fungi.

The following fungi were tested: *Trichoderma lignorum*, *Acremoniella olivaespora*, *Aspergillus niger*, *A. terreus*, *A. nidulans*.

The fungi tested were obtained from the stock cultures of the Mycology Department (isolated from the Rothamsted soils). They were maintained on agar slants in Waksman's medium. Before inoculation they were always grown afresh and in all the experiments described below cultures one week old were used.

Estimations of ammonia-N, total N, extraction with 90 % alcohol and treatment with hydrogen peroxide were carried out as in Part I.

(1) *Pentose units* (total furfuraldehyde). The standard method of Krober and Tollens was employed. The phloroglucide precipitate was not extracted with alcohol.

(2) *Cellulose* was estimated by Jenkins's method [1930].

(3) *Furfuraldehyde in cellulose* was determined as in (1).

The loss of dry matter (D.M.) as a result of decomposition is usually not a true index of the amount of decomposition. It does not consider the synthesis of new complexes by micro-organisms, which would modify to a considerable extent the actual amount of material decomposed. The cellulose and furfuraldehyde determinations would indicate how much of the loss in organic matter is represented by the loss in cellulose and hemicelluloses.

In any mature plant material, the cellulose, the polysaccharide associated with cellulose, or as it is termed by Hawley and Norman [1932], the "cellulosan," and the hemicelluloses are the three chief groups of biologically available material. The cellulose as isolated either by the Cross and Bevan chlorination method or by the Jenkins hypochlorite method is always associated with cellulosan. This cellulosan generally consists of pentose units. In the case of oat straw it is xylan as proved by Norman [1929]. This xylan can be determined by distilling the cellulose with 12 % HCl. A figure for "true" cellulose can then be obtained by deducting the figure for xylan. It is found that the decompositions of cellulose and the xylan associated with it run parallel. A figure for pentose units in the hemicelluloses can be obtained by subtracting from the total yield of furfuraldehyde that from the xylan associated with cellulose.

For the sake of comparison the figures recorded below are calculated on a basis of 100 g. of dry straw and interpreted in terms of cellulose, xylan associated with cellulose and pentose in hemicelluloses.

EXPERIMENTAL.

The same sample of oat straw was used throughout these experiments with pure cultures of organisms.

	%
Ash	9·04
Total N	0·35
Total furfuraldehyde	15·6
"True" cellulose	41·0
Furfuraldehyde on Jenkins's cellulose product	7·0
Xylan associated with cellulose	10·8
Furfuraldehyde due to pentose in polyuronides	8·6
Alcohol extract	3·46

Series I (Tables I and II).

Stickiness. No stickiness was observed with any fungi used for decomposition.

Carbohydrate constituents. Table I contains analyses of these constituents. *Acremoniella sp.* appears to be the most active fungus as judged from the loss of dry matter at the end of 48 days. *T. lignorum* seems to be the least active. The losses obtained follow practically the same order as those recorded by Norman [1931]. In general the hemicelluloses suffer a loss of about 60 % during 48 days. The hemicelluloses appear to decompose rapidly in the first few days and then remain at much the same level while the cellulose is being decomposed. On the whole it is obvious that the major part of the lost carbonaceous material is accounted for by the cellulose which is a more abundant food and energy source for the micro-organisms than the hemicelluloses.

Table I. *Decomposition of straws by various fungi at 35° at different intervals.*

Expressed on 100 g. original straw.

Fungus	Time in days	Loss of D.M.	Furfuraldehyde from pentose groups not in cellulose	"True" cellulose	Loss of "true" cellulose	Loss of furfuraldehyde from non-cellulosic constituents
Acre. olivaespora	8	10·9	6·37	36·90	4·1	2·23
	16	21·2	5·19	27·81	13·1	3·41
	24	28·1	4·56	23·65	17·3	4·04
	48	41·2	3·32	16·17	24·8	5·28
A. nidulans	8	9·2	7·04	38·85	2·1	1·56
	16	15·6	6·01	35·64	5·3	2·59
	24	31·5	3·87	22·42	18·5	4·73
	48	38·9	3·23	17·02	23·9	5·37
A. terreus	8	11·0	6·98	37·66	3·3	1·62
	16	15·9	5·22	31·23	9·7	3·38
	24	31·9	4·66	24·09	16·9	3·94
	48	34·6	4·01	19·88	21·1	4·59
A. niger	8	11·2	4·98	37·37	2·6	3·62
	16	15·8	5·38	34·27	7·1	3·22
	24	23·1	4·86	26·25	14·7	3·74
	48	34·1	3·67	23·13	17·8	4·93
T. lignorum	8	9·3	4·23	38·29	2·7	4·37
	16	16·0	5·08	35·08	5·9	2·80
	24	24·4	5·02	27·82	13·1	3·58
	48	29·0	4·17	23·36	16·6	4·43

Table II. *Nitrogen content of straws rotted by pure cultures of fungi at 35° at different intervals.*

Expressed on 100 g. original straw.

Fungus	Time in days	Loss of D.M.	Total N	Organic N	N factor	N equiv.
Acre. olivaespora	8	10·9	1·37	0·98	0·63	5·7
	16	21·2	1·32	1·29	0·93	4·4
	24	28·1	1·24	1·21	0·85	3·0
	48	41·2	1·19	1·17	0·84	2·0
A. nidulans	8	9·2	1·14	0·62	0·27	2·9
	16	15·6	0·92	0·72	0·37	2·3
	24	31·5	0·94	0·92	0·56	1·8
	48	38·9	1·12	1·09	0·73	1·8
A. terreus	8	11·0	1·22	0·75	0·40	3·7
	16	15·9	1·12	0·99	0·64	4·0
	24	31·9	0·85	0·82	0·51	1·6
	48	34·6	1·02	1·01	0·66	1·9
A. niger	8	11·2	1·20	0·82	0·46	4·1
	16	15·8	1·11	1·03	0·65	4·1
	24	23·1	1·24	1·22	0·86	3·7
	48	34·1	1·02	1·02	0·86	2·5
T. lignorum	8	9·3	1·23	0·86	0·50	5·4
	16	16·0	1·09	0·93	0·65	4·8
	24	24·4	1·25	1·24	0·88	3·6
	48	29·0	1·25	1·24	1·01	3·4

Nitrogen immobilisation. From Table II it can be seen that more than 50 % of the inorganic nitrogen has been used up by the organisms except *A. nidulans* in the first 8 days. The power of an organism in building up the microbial protein can best be judged from the nitrogen factor [Richards and Norman, 1931]. *T. lignorum* seems to be the best from the point of view of nitrogen immobilisation and *A. nidulans* the poorest. Norman [1931] also recorded a low nitrogen factor for *A. nidulans* compared with *A. niger* and *A. terreus*. As compared by their nitrogen equivalents [Norman, 1931] *A. nidulans* seems to be the most efficient and *Trichoderma* the least. *A. niger* differs markedly from *A. nidulans* and *A. terreus* though belonging to the same genus.

Series II (Tables III, IV and V).

Stickiness. Both the organisms produce a negligible amount of stickiness. The figures for *S. cytophaga* are contrary to expectation. Since it is an organism which produces a gummy colony on agar media, it was supposed that it might also give a rotted product showing stickiness.

Table III. *Physical test, alcoholic extracts and the H_2O_2 treatment of straws rotted with pure cultures of bacteria at 35° at different intervals.*

Expressed on 100 g. dry manure.

Bacterium	Time in days	Loss of D.M.*	Alcohol extract	H_2O_2 extract	Loss of O.M. after H_2O_2 treatment	Physical test (g.)
S. cytophaga	8	7·5	8·40	7·0	10·1	108·6
	16	12·2	6·87	10·1	11·4	108·2
	24	13·0	7·48	13·9	11·4	212·3
	48			Infected		
M. agreste	8	4·8	7·05	13·7	12·6	101·3
	16	8·5	7·41	12·1	11·4	0·0
	24	14·2	7·51	9·7	13·8	334·1
	48			Infected		

* On 100 g. original straw.

Extractions with alcohol and hydrogen peroxide (Table III). On an average the alcoholic extract is the same for both of the organisms with no further change in the degree of decomposition. There is an increase in the hydrogen peroxide extract with *S. cytophaga* whereas the order is reversed with *M. agreste*.

Carbohydrate constituents (Table IV). The decomposition is practically of the same order with both the organisms at the end of 24 days. The bottles for the 48th day were infected and hence rejected. The cellulose lost with *Mycobacterium* is nearly one and a half times that destroyed by *Spirochaeta*. This was entirely unexpected, since the former is not ordinarily regarded as being a cellulose-decomposing organism and does not develop on a cellulose-agar plate, but, as pointed out by Norman [1930], the ability to utilise cellulose in the presence of other available carbohydrates is more common than is generally supposed. On the other hand *Spirochaeta* destroys hemicelluloses to a considerably greater extent than does *Mycobacterium*. Both organisms compare poorly with fungi in destructive action as regards organic matter.

Table IV. *Decomposition of straw by bacteria at 35° at different intervals.*

Expressed on 100 g. original straw.

Bacterium	Time in days	Loss of D.M.	Furfuraldehyde from pentose groups not in cellulose	"True" cellulose	Loss of "true" cellulose	Loss of furfuraldehyde from non-cellulosic constituents
S. cytophaga	8	7·5	5·60	38·82	2·1	3·00
	16	12·2	6·11	36·76	4·2	2·59
	24	13·0	5·31	36·29	4·7	3·29
	48			Infected		
M. agreste	8	4·8	6·06	39·14	1·8	2·54
	16	8·5	6·02	36·43	4·5	2·58
	24	14·2	5·95	34·31	6·6	2·65
	48			Infected		

Nitrogen immobilisation. The nitrogen figures are recorded in Table V. *Mycobacterium* builds up more microbial tissue than does *Spirochaeta* as seen from the nitrogen factor. Compared with fungi, bacteria appear to be much slower in building up microbial protein. *Spirochaeta* consumes more carbohydrate per g. of nitrogen than *Mycobacterium* as shown by the nitrogen equivalent.

Table V. *Nitrogen content of straw rotted with pure cultures of bacteria at 35° at different intervals.*

Expressed on 100 g. original straw.

Bacterium	Time in days	Loss of D.M.	Total N	Organic N	N factor	N equiv.
S. cytophaga	8	7·5	1·268	0·75	0·39	5·2
	16	12·2	1·000	0·64	0·29	2·3
	24	13·0	0·957	0·80	0·46	3·5
				Infected		
M. agreste	8	4·8	1·290	0·93	0·58	11·8
	16	8·5	1·040	0·66	0·30	3·5
	24	14·2	1·180	0·97	0·60	4·2
				Infected		

Table VI. *Decomposition of straw rotted first with pure cultures of fungi for 48 days and then inoculated with* M. agreste *and analysed at different intervals.*

Expressed on 100 g. original straw.

Fungus	Days after inoculation	Loss of D.M.	Furfuraldehyde from pentose groups not in cellulose	"True" cellulose	Loss of "true" cellulose	Loss of furfuraldehyde from non-cellulosic constituents
T. lignorum	8	30·1	3·52	24·74	16·2	5·08
	16	44·5	2·34	12·92	28·0	6·26
	24	49·1	2·46	11·86	29·1	6·14
	48	39·0*	3·61	21·14	19·8	5·00
A. niger	8	35·7	3·74	20·20	20·8	4·86
	16	42·0	3·04	15·99	25·0	4·56
	24	36·0	3·63	17·52	23·4	4·97
	48	41·0	3·06	17·70	23·3	4·74
A. nidulans	8	25·0	4·39	29·53	11·4	4·21
	16	28·0	4·26	27·38	12·6	4·34
	24	36·0	2·94	18·98	22·0	5·66
	48	38·7	3·79	20·96	20·0	4·81
Acre. olivaespora	8	32·4	4·06	25·18	15·8	4 54
	16	43·3	3·14	16·48	24·5	5·46
	24	43·4	2·94	15 59	25·4	5·66
	48	44·5	3·60	18·32	22·6	5·00
A. terreus	8	31·2	3·57	23·12	17·8	5·03
	16	36·9	3·55	19 00	22·0	5·03
	24	37·5	3·91	21·19	19·8	4·69
	48	39·7	3·42	19·81	21·2	5·18

* Short of moisture.

Table VII. *Nitrogen content of straw rotted first with pure cultures of fungi for 48 days and then inoculated with* M. agreste *and analysed at different intervals.*

Expressed on 100 g. original straw.

Fungus	Days after inoculation	Loss of D.M.	Total N	Organic N	N factor	N equiv.
T. lignorum	8	30·1	1·01	1·00	0·58	3·3
	16	44·5	1·22	1·21	0·70	1·5
	24	49·1	1·14	1·10	0·77	1·5
	48	39·0*	0·91	0·88	0·55	1·4
A. niger	8	35·7	1·08	1·06	0·72	2·0
	16	42·0	1·25	1·19	0·84	2·0
	24	38·0	0·95	0·94	0·67	1·7
	48	41·0	0·92	0·90	0·54	1·3
A. nidulans	8	25·0	0·97	0·95	0·62	2·5
	16	28·4	0·97	0·96	0·60	2·1
	24	36·9	1·16	1·14	0·80	2·1
	48	38·7	0·97	0·95	0·60	1·5
Acre. olivaespora	8	32·4	1·00	0·98	0·70	2·1
	16	43·3	1·25	1·19	0·84	1·9
	24	43·4	1·19	1·13	0·82	1·9
	48	44·5	1·10	1·08	0·73	1·6
A. terreus	8	31·2	1·06	1·05	0·69	2·2
	16	36·9	0·97	0·96	0·59	1·6
	24	37·5	0·87	0·86	0·51	1·3
	48	39·7	0·95	0·88	0·53	1·3

* Short of moisture.

Series III (*a*) (Tables VI and VII).

Stickiness. The rots showed no stickiness, indicating that *M. agreste* is unable to synthesise sticky material even after acting upon the elaborated fungal tissue.

Carbohydrate constituents (Table VI). *M. agreste* following upon *Trichoderma* gives the best decomposition and a loss of 49·1 % organic matter in only 24 days. The losses with the *Aspergilli* are practically of the same order. The rate of decomposition in the first eight days after inoculation with all the fungi is greater than in any subsequent periods. The average loss of cellulose with *Mycobacterium* following upon the five different fungi ranges from 20 to 23 %. Further decomposition does not affect the pentose unassociated with cellulose showing that all the available pentoses have already been removed by the fungi.

Nitrogen immobilisation (Table VII). With *Mycobacterium* following on each of the fungi, there is a drop in the nitrogen factor after the 16th day in general, which is due to the ammonification or as it is termed by Jensen [1929] the "mineralisation" of a portion of the fungal protein.

Series III (*b*) (Tables VIII, IX and X).

Stickiness. The results for stickiness are given in Table VIII and are highly significant. No stickiness was found with fungi on the 48th day, but by the subsequent action of *S. cytophaga* for 8 days only, the pull required to separate the two plates was at once raised by from 3 to 6 kg. varying with the nature of the fungus. This obviously means that *Spirochaeta* has synthesised some organic matter which is responsible for the stickiness. Since it was observed in Series II that *Spirochaeta* alone could hardly produce any stickiness, one is naturally led

Table VIII. *Physical test, alcohol extracts and the H_2O_2 treatment of straws rotted first with pure cultures of fungi and then inoculated with* S. cytophaga *and analysed at different intervals.*

Expressed on 100 g. dry manure.

Fungus	Days after inoculation	Loss of D.M.	Alcohol extract	H_2O_2 extract	Loss of O.M. with H_2O_2	Physical test (g.)
T. lignorum	8	39·2	10·50	13·1	22·0	3495
	16	39·7	8·50	12·1	22·3	6435
	24	42·6	8·87	12·8	23·0	3958
	48	57·7	12·20	29·0	26·0	6550
A. niger	8	41·4	9·90	13·3	24·7	6305
	16	45·0	8·90	17·5	25·6	4242
	24	46·0	10·20	26·6	24·7	7134
	48	48·3	9·20	24·7	25·0	6145
A. nidulans	8	35·8	8·80	11·7	22·8	5197
	16	41·2	8·60	16·8	26·2	6796
	24	43·4	9·04	13·8	30·0	5869
	48	43·5	9·05	22·8	22·0	5290
Acre. olivaespora	8	43·1	9·14	13·3	24·0	4425
	16	43·2	8·02	13·8	23·2	7866
	24	45·0	8·56	21·0	25·0	4995
	48	46·3	7·64	15·0	25·0	6904
A. terreus	8	37·5	7·64	14·2	23·7	6439
	16	41·9	7·90	11·8	26·4	5970
	24	45·4	7·64	15·0	25·0	8386
	48	51·3	7·82	26·4	25·0	6442

The final p_H of all the samples was between 7·0 and 7·5.

to believe that the sticky material has some relation to the previous action of the fungus. Again, *Mycobacterium* did not produce any stickiness even after a previous fungal decomposition, suggesting that the production of stickiness has also some relation to the nature of the bacterium taking part in the decomposition.

Extractions by alcohol and hydrogen peroxide (Table VIII). The averages for the alcohol extracts differ in each case, the maximum being 10 % with *Spirochaeta* following on *Trichoderma* and 7·7 % on *A. terreus*. These figures are interesting when compared with stickiness. The maximum stickiness obtained is with the latter combination, whereas the former gives the least. On the whole the alcoholic extracts are greater than those with *Mycobacterium*. The higher solubility of the sticky material in alcohol indicates its possible non-gummy and non-dextrin nature. The losses of organic matter on treatment with peroxide are in general proportional to the losses in dry matter.

Carbohydrate constituents (Table IX). Greatest decomposition is obtained by *Spirochaeta* following on *Trichoderma* and *A. terreus* and amounts to 51·7 and 51·3 % respectively. In these two cases there is a big jump in the loss of dry matter between the 24th and 48th days after the inoculation with *Spirochaeta*, but with the remainder the decomposition appears to be steady. The average loss of cellulose is about 26 % in each case. The average loss of pentose not associated with cellulose is also practically the same. These losses are not significantly higher than those given by fungi alone at the end of 48 days, indicating that no available hemicelluloses are left after fungus action.

Table IX. *Decomposition of straws rotted first with pure cultures of fungi for 48 days and then inoculated with* S. cytophaga *and analysed at different intervals.*

Expressed on 100 g. original straw.

Fungus	Days after inoculation	Loss of D.M.	Furfuraldehyde from pentose groups not in cellulose	"True" cellulose	Loss of furfuraldehyde from non-cellulosic constituents
T. lignorum	8	39·2	3·16	14·61	5·24
	16	39·7	3·06	18·26	5·54
	24	42·6	3·17	14·89	5·43
	48	51·7	2·21	10·58	6·39
A. niger	8	41·4	2·88	14·57	5·72
	16	45·0	2·44	13·78	6·16
	24	46·0	2·56	13·52	6·04
	48	48·3	2·39	12·03	6·21
A. nidulans	8	35·8	3·88	18·46	4·72
	16	41·2	2·89	13·65	5·71
	24	43·4	3·09	16·28	5·51
	48	43·5	2·94	13·89	5·66
Acre. olivaespora	8	43·1	2·86	13·79	5·74
	16	43·2	3·41	15·85	5·19
	24	45·0	2·66	13·42	5·94
	48	46·3	2·20	13·06	6·40
A. terreus	8	37·5	3·15	16·45	5·45
	16	41·9	2·86	14·61	5·74
	24	45·4	2·20	13·74	6·40
	48	51·3	2·06	12·58	6·54

Nitrogen immobilisation (Table X). Here again there is a drop in the nitrogen factor as the decomposition proceeds. This fall is more marked with *Spirochaeta* than with *Mycobacterium*. The former appears more efficient than the latter per unit of nitrogen as judged by the nitrogen equivalent.

Table X. *Nitrogen content of straws rotted first with fungi for 48 days and then inoculated with* S. cytophaga *and analysed at different intervals.*

Expressed on 100 g. original straw.

Fungus	Days after inoculation	Loss of D.M.	Total N	Organic N	N factor	N equiv.
T. lignorum	8	39·2	1·26	1·24	0·89	2·2
	16	39·7	1·13	1·11	0·77	1·8
	24	42·6	1·10	1·08	0·73	1·7
	48	51·7	1·12	1·09	0·74	1·4
A. niger	8	41·4	1·35	1·30	0·96	2·3
	16	45·0	1·24	1·18	0·82	1·8
	24	46·0	1·26	1·18	0·83	1·8
	48	48·3	1·20	1·11	0·76	1·5
A. nidulans	8	35·8	1·22	1·20	0·91	2·5
	16	41·2	1·39	1·31	0·84	2·0
	24	43·4	1·14	1·12	0·77	1·7
	48	43·5	1·16	1·13	0·80	1·8
Acrc. olivaespora	8	43·1	1·31	1·23	0·95	2·1
	16	43·2	1·15	1·11	0·78	1·8
	24	45·0	1·13	1·10	0·76	1·7
	48	46·3	1·25	1·23	0·82	1·7
A. terreus	8	37·5	1·34	1·27	0·99	2·6
	16	41·9	1·28	1·25	0·85	2·0
	24	45·4	1·23	1·19	0·84	1·7
	48	51·3	1·20	1·17	0·81	1·6

Series IV (Tables XI and XII).

Stickiness (Table XI). Fungi alone produce no stickiness, but it develops as soon as *S. cytophaga* is inoculated even when the fungus had a start of only a week. This further emphasises the necessity of the presence of fungal tissue for the production of stickiness. The stickiness was practically constant at about 4275 g. when *Spirochaeta* acted for only 8 days whereas it varied considerably when it acted for 40 days.

Table XI. *Decomposition of straw rotted first with fungus for different periods and then inoculated with* S. cytophaga *and analysed on the 8th and 40th days after inoculation.*

Expressed on 100 g. original straw.

Organism	Days of fungus action	Loss of D.M.	Furfuraldehyde from pentose groups not in cellulose	"True" cellulose	Loss of "true" cellulose	Loss of furfuraldehyde from non-cellulosic constituents	Physical test (g.)
T. lignorum	8	27·8	4·27	23·52	17·4	3·33	3771
+	16	36·5	2·93	16·60	24·4	5·67	4452
S. cytophaga	24	37·6	3·36	14·10	26·9	5·24	4735
for 8 days	48	48·5	2·18	9·30	31·7	6·42	4143
T. lignorum	8	46·2	2·74	10·24	30·7	5·86	5196
+	16	47·9	2·32	9·45	31·5	6·28	1998
S. cytophaga	24	48·9	1·76	10·03	30·9	6·94	1880
for 40 days	48	54·9	1·36	7·97	33·0	7·24	2077

Carbohydrate constituents (Table XI). By comparing the figures in Series I for losses in dry matter with those of *Trichoderma* alone in Table I an estimate can be obtained as to the further decomposition due to *Spirochaeta* in conjunction with the fungus. For instance, the fungus working alone decomposed 16 % of dry matter in 16 days, whereas if working in association with the bacterium for the second 8 days, *i.e.* a total of 16 days, a decomposition of 27 % was obtained.

Obviously this extra loss must be ascribed to the action of the bacterium. For 48 days with the fungus and 8 days with the bacterium the decomposition amounted to 48 %, which is very close to that obtained with the fungus for 16 days and the bacterium for 40 days. The loss of dry matter for 48 days with the fungus and 40 days with the bacterium is about 55 %, which compares well with 51 % obtained in Series III (*b*) for a total period of 96 days. The losses in carbohydrate constituents run parallel with the losses in dry matter.

Nitrogen immobilisation (Table XII). The increase in ammonification with the period of incubation explains the drop in the nitrogen factor. *Spirochaeta* has accordingly decomposed the fungus protein for its nitrogen requirements; the excess of ammonia is volatilised, thus accounting for the nitrogen losses.

Table XII. *Nitrogen content of straws rotted first with fungus for different periods and then analysed on the 8th and 40th days after inoculation with* S. cytophaga.

Expressed on 100 g. original straw.

Organism	Days of fungus action	Loss of D.M.	Total N	Organic N	N factor	N equiv.
T. lignorum	8	27·8	1·49	1·45	1·09	3·9
+	16	36·5	1·36	1·21	0·86	2·3
S. cytophaga	24	37·6	1·32	1·18	0·82	2·2
for 8 days	48	48·5	1·29	1·23	0·89	1·8
T. lignorum	8	46·2	1·33	1·30	0·94	2·0
+	16	47·9	1·38	1·34	0·98	2·0
S. cytophaga	24	48·9	1·30	1·23	0·88	1·8
for 40 days	48	54·9	1·21	1·16	0·81	1 5

Series V (Tables XIII and XIV).

Stickiness. The figures for stickiness indicate that the joint action of the two organisms from the very start is unfavourable for its production. The average of 240 g. for stickiness is far below the figures recorded where the fungus had acted alone for at least a week. The low value for stickiness may be accounted for by the failure of the fungus to build up the microbial tissue which appears to be of prime importance in the production of stickiness.

Carbohydrate constituents (Table XIII). The rate of decomposition appears to be very steady, giving a maximum of about 28 % which is approximately that of *Trichoderma* alone for 48 days. In fact, greater decomposition was expected considering the cellulose-decomposing nature of both the organisms. This low decomposition may partly be accounted for by the apparent failure of the fungus to grow in presence of *Spirochaeta.* The cellulose loss is in proportion to the loss of dry matter. A loss of 13 % dry matter between the 24th and 48th days

Table XIII. *Decomposition of straw with fungus and* S. cytophaga *together for different intervals.*

Expressed on 100 g. original straw.

Organism	Time in days	Loss of D.M.	Furfuraldehyde from pentose groups not in cellulose	"True" cellulose	Loss of "true" cellulose	Loss of furfuraldehyde from non-cellulosic constituents	Physical test (g.)
T. lignorum	8	6·9	6·69	37·93	3·0	1·91	139
+	16	11·9	6·52	35·41	5·5	2·08	163
S. cytophaga	24	14·5	5·02	33·77	7·2	3·58	395
together	48	27·9	4·68	20·62	20·3	3·92	266

corresponds with 13 % loss of cellulose during the same period. The greater part of the loss of organic matter is thus accounted for by the loss in cellulose. There is hardly any increase in the loss of pentose between 24 and 48 days, no doubt due to the inactivity of the fungus.

Table XIV. *Nitrogen content of straws rotted with fungus and* S. cytophaga *together for different intervals.*

Expressed on 100 g. original straw.

Organism	Time in days	Loss of D.M.	Total N	Organic N	N factor	N equiv.
T. lignorum	8	6·9	1·37	0·95	0·60	8·6
+	16	11·9	1·28	1·00	0·64	5·4
S. cytophaga	24	14·5	1·04	0·84	0·48	3·3
together	48	27·9	1·30	1·27	0·92	3·3

Nitrogen immobilisation (Table XIV). The organic nitrogen is lower than that obtained with the fungus alone. This may be explained by the inactivity of the fungus. The low nitrogen factor in the early stages may be due to the poor synthesis of fungal tissue in the presence of the bacterium.

DISCUSSION AND CONCLUSIONS.

It appears from these decomposition studies that the production of stickiness in straws depends upon the presence of fungal tissue and the nature of the bacterium. Since *S. cytophaga* has a comparatively low optimum temperature for development, it appears that under the high temperature conditions of fermentation in a manure heap some other organisms must be the main causes of production of stickiness. Stickiness seems to have no correlation with the disappearance of any particular carbohydrate constituent during decomposition.

Although *S. cytophaga* produces gum on a synthetic medium, it fails to produce any stickiness while working upon straws, but in the presence of fungal tissue *S. cytophaga* produces stickiness. It is possible therefore that there is a fundamental difference between the sticky material synthesised by *S. cytophaga* while working upon straw previously decomposed by fungi and the gum it produces on an artificial medium which contains no fungal tissue. Obviously some sticky material other than the bacterial gum is synthesised during decomposition of straw by the interaction between the fungal tissue and the bacterium.

The simultaneous inoculation of fungus and *S. cytophaga* on sterile straw was not very successful either from the point of view of production of stickiness or of general decomposition. The fungus developed with difficulty and two further inoculations were necessary. This may be due either to competition for food material between the fungus and the bacterium or the retarding effect of the end-products of the bacterial action on the growth of the fungus. No counts were made to test this possibility, but it has been observed by Rege [1927] and others that fungi which bring about the initial decomposition of straw eventually disappear and are replaced by bacteria.

SUMMARY.

(1) A number of common soil fungi and two cellulose-decomposing bacteria in pure culture and in different associations have been tested with reference to the production of stickiness and general decomposition.

(2) These fungi and bacteria, while working independently of each other, do not produce stickiness irrespective of the nature of the bacteria.

(3) Fungus decomposition followed by the action of *Mycobacterium agreste* does not produce stickiness.

(4) Fungus decomposition followed by the action of *Spirochaeta cytophaga* produces stickiness.

(5) Progressive decomposition with a fungus and subsequent inoculation with *S. cytophaga* at different stages produce stickiness even if the period of action of the fungus was brief.

(6) Simultaneous inoculations of fungus and *S. cytophaga* produce very little stickiness.

(7) The amount of decomposition effected, the losses in carbohydrate constituents and the nitrogen immobilisation in each case were determined. All the substances studied were removed approximately in proportion to the apparent losses of dry matter.

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STUDIES ON CALCIUM CYANAMIDE[1].

I. THE DECOMPOSITION OF CALCIUM CYANAMIDE IN THE SOIL AND ITS EFFECTS ON GERMINATION, NITRIFICATION AND SOIL REACTION.

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(With Eight Text-figures.)

Calcium cyanamide was one of the first synthetic nitrogenous fertilisers, and it still remains the cheapest per unit of nitrogen. The first large-scale plant was completed in 1905, and there is no evidence that the process is being displaced by the newer direct ammonia synthesis. The production increased from 34,000 tons of nitrogen in 1913, or 4·5 per cent. of the total world's supply, to 264,000 tons of nitrogen in 1928–9 which formed 12 per cent. of the total supply and 26 per cent. of that from synthetic processes. Cyanamide plants have been erected, partly for reasons of national defence, in no less than eleven European countries as well as in North America and Japan. Its consumption has, however, remained more localised and specialised than that of the other nitrogenous fertilisers. It is used extensively in Germany, Poland, Holland and Belgium, but has made comparatively little progress in this country. In the United States until recently it was employed only as a drier and conditioner in mixed fertilisers at a rate of about 2·5 per cent., but now its direct use is developing, especially in the southern States.

The relatively low price per unit of nitrogen depends in part on the fact that it is the only fertiliser produced directly by a synthetic process.

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[1] Several past and present members of the Rothamsted staff collaborated in the work described in this series of papers and, although it proved impossible to publish the contributions separately, the names of those responsible for the major experiments are given in the text, tables or figures. The series of experiments by the late A. J. Walker were planned under the direction of H. J. Page, formerly Head of this Department. Subsequent papers deal with: (II) The microbiological aspects of nitrification in soils under varied environmental conditions (B. K. Mukerji); (III) Storage and mixing with superphosphate (H. L. Richardson); (IV) The utilisation of calcium cyanamide in pot and field experiments on arable crops (H. L. Richardson and E. M. Crowther); and (V) The use of calcium cyanamide and other forms of nitrogen for grassland (H. L. Richardson).—E.M.C.

Other methods yield ammonia or oxides of nitrogen, and although anhydrous ammonia is cheaper per unit of nitrogen, the necessity for combining it with a suitable carrier makes ammonia nitrogen more expensive than cyanamide nitrogen. Cyanamide also has the advantage that it is the only nitrogenous fertiliser containing excess lime, and recent patent literature shows that considerable attention is being given to methods for converting synthetic ammonia into "white calcium cyanamide" in order to secure a cheap basic nitrogen carrier. In spite of these advantages calcium cyanamide has made relatively slow progress in many countries, partly through its unusual appearance and unpleasantness in handling and partly through untoward results from inadequate understanding of its special properties and its limitations. In comparative trials against other nitrogenous fertilisers it is generally compared under identical conditions with no attempt to ascertain whether these are suitable for its effective use. In all comparisons of a relatively new and little-known product against old and well-established ones the less known is necessarily at a disadvantage.

The work to be described in the present series of papers was undertaken at Rothamsted with the object of securing a better understanding of the use of cyanamide based on the study of its behaviour in the soil and its effect on crops under a range of practical and experimental conditions. Apart from their bearing on the agricultural use of cyanamide the papers deal with the complex interactions of the soil colloids, the soil micro-organisms and the growing plant which follow the addition of such a highly reactive substance as calcium cyanamide.

The early work of Kappen (1), Ulpiani (2) and others established the nature of the essential changes in the soil of cyanamide to urea and then through ammonia to nitrate with some delay in nitrification as compared with ammonium sulphate, and early experience demonstrated the toxicity of cyanamide or its immediate products to germinating seeds and the green leaf. In a large measure our own work confirms and extends these early findings for a range of soil and weather conditions, and in addition it attempts to ascertain how far the special characteristics of calcium cyanamide may be definitely exploited in agriculture. A re-examination of the earlier work was required not only to relate it to British conditions and to clear up some of the highly conflicting statements in the literature but also to ascertain whether the post-War product behaved similarly to the pre-War one. It was often stated that the troubles encountered in the early days of the cyanamide industry were to be ascribed to irregularities of manufacture or storage and to the unsatisfactory methods adopted for counteracting the excessive dustiness of the product from the furnaces. Since the War this has been met by the incorporation of small amounts of heavy oil, and the final product is free from some of the impurities or decomposition products often found in the earlier granulated form.

The relative values of ammonium sulphate and calcium cyanamide have too often been assessed from laboratory nitrification experiments on the assumption that plants assimilate nitrogen only as nitrate, and many adverse judgments on calcium cyanamide are based on this invalid criterion. The slow formation of nitrate from calcium cyanamide has sometimes been explained by the slowness of the initial changes to urea and ammonia, but it will be shown that calcium cyanamide decomposes very rapidly in most soils. It has, however, a direct influence on the nitrifying organisms. For these reasons the work was planned to re-examine from the beginning each of the stages in its decomposition in the soil with the object of developing our knowledge of the material and the conditions for its effective use, rather than in the hope of estimating its average value relative to other and better known products.

It is unnecessary to review the earlier literature, for satisfactory summaries are available in the works of Pranke (3) and Prianischnikow (4) (general accounts), in Honcamp's recent *Handbuch der Dungerlehre* (5) (chemistry of cyanamide and technical aspects of its manufacture), and in papers by Buchanan and Barsky (6) (decomposition in solutions) and by Jacob, Allison and Braham (7) (nitrification in soils under laboratory conditions). Only such points as are needed for understanding the analytical methods and the behaviour of calcium cyanamide in the soil or in mixtures with other fertilisers are given in the present papers.

THE CHEMISTRY OF CALCIUM CYANAMIDE AND CYANAMIDE.

To avoid confusion, the name of the commercial product will be written as "Calcium Cyanamide," and that of the pure salt without capitals; the free acid will be termed "free cyanamide" and the word "cyanamide" will be used as a general term, when the precise mode of occurrence is unknown or immaterial.

As generally manufactured, Calcium Cyanamide contains 63 per cent. calcium cyanamide or 22 per cent. of nitrogen, 13 per cent. of carbon, and 20 per cent. of quicklime. It is adjusted by additions of quicklime to the various grades favoured by the markets of different countries (*e.g.* 20·6 per cent. nitrogen in the British Isles). Small amounts of calcium carbide and oil are also present.

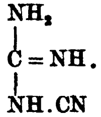
Calcium cyanamide, which is present in the fertiliser in a crystalline form, dissolves with decomposition in water to give an acid salt and calcium hydroxide:

$$2CaCN_2 + 2H_2O \rightarrow Ca(HCN_2)_2 + Ca(OH)_2.$$

In concentrated solutions a basic salt ultimately separates out in needles and free cyanamide is formed:

$$2Ca(HCN_2)_2 + 2H_2O \rightarrow (CaOH)_2CN_2 + 2H_2CN_2.$$

Free cyanamide may be regarded either as $H_2N.CN$ or as carbodiimide $HN{=}C{=}NH$, and both forms are probably present in solution. It behaves as a weak monobasic acid with a dissociation constant of $5{\cdot}4 \times 10^{-11}$ at 25°; dibasic salts do not exist in solution. It precipitates an insoluble silver salt (Ag_2CN_2) from alkaline silver nitrate solutions, the precipitate being also insoluble in ammonia. (This property is made use of in its analysis in the determination of cyanamide nitrogen and its separation from dicyanodiamide.)

An aqueous solution of pure cyanamide is relatively stable, but in the presence of acid or alkali or certain other catalysts it undergoes fairly rapid changes. In moderately *alkaline solutions*, especially when heated, it polymerises almost quantitatively to dicyanodiamide  $\overset{NH_2}{\underset{NH.CN}{C}}{=}NH$.

The rate of reaction increases with the *p*H value up to about 9·6, but in still more alkaline conditions (*p*H greater than 10) the rate falls off rapidly, whilst hydrolysis to urea commences and becomes almost quantitative about *p*H value 12. These changes take place naturally in Calcium Cyanamide when it is allowed to become moist (on storage), and dicyanodiamide may be produced almost quantitatively by boiling an aqueous suspension of Calcium Cyanamide which is rendered alkaline by its own CaO content. Dicyanodiamide is a soluble crystalline substance which does not form salts with either acids or alkalis. From an alkaline silver solution it precipitates a silver derivative, which is soluble in ammonia (contrast cyanamide, above). Heated with acid it hydrolyses to the strong base, guanylurea, which forms an insoluble nickel derivative. These properties are used in its determination (see Appendix).

In *acid solutions* free cyanamide is hydrolysed to urea, and the reaction is catalysed by many inorganic compounds, especially salts or oxides of iron or manganese. This is the normal change undergone by cyanamide in the soil.

The influence of reaction on the decomposition of cyanamide is of some practical importance in connection with its behaviour when mixed with superphosphate. During this mixing heat is generated, and in the presence of the moisture from the superphosphate rapid changes are possible. Their exact nature varies with the proportions of the two substances, and the resultant reaction of the mixture; with a small proportion of Calcium Cyanamide, insufficient to neutralise the acidity of the acid calcium phosphate, urea predominates. But with increasing proportions of Calcium Cyanamide the mixture becomes more and more alkaline, and the percentage of the cyanamide nitrogen polymerised to dicyanodiamide increases rapidly to a maximum and then falls off (15)

The above reactions are often accompanied by side reactions which made quantitative recoveries and exact interpretation difficult. Unfavourable results in the use of Calcium Cyanamide have often been ascribed on inadequate evidence to the presence or production of dicyanodiamide, and, although it is extremely difficult to follow exactly the changes in soils, we shall present evidence to show that some of these effects may more properly be ascribed to cyanamide itself, or to some product whose decomposition follows that of cyanamide so closely that it becomes of purely academic interest to attempt to distinguish between them.

THE DISAPPEARANCE OF CYANAMIDE FROM THE SOIL.

In the early days of the industry it was believed that micro-organisms were responsible for the change from cyanamide to ammonia in the soil. Although some bacteria and fungi are able to attack cyanamide, many inorganic catalysts for the cyanamide to urea reaction were discovered by Ulpiani, Kappen and others. Oxides, hydroxides and ores of iron and manganese and certain zeolites proved the most active agents, but animal charcoal was also highly active. Cowie (8) showed in this laboratory that in sterile soils urea was rapidly formed and accumulated; in partially sterilised soils the urea disappeared again after a few days and ammonia accumulated, and in normal soils the urea disappeared still more rapidly and ammonia and nitrates accumulated. Even for dressings several times heavier than those used in the field one-half or more of the cyanamide was converted to urea and ammonia within 1 or 2 days in normal agricultural soils, whether sands, loams, or clays; in poor heath sands and in some highly organic fen soils the decomposition proceeded more slowly. A quartz sand from a deep pit was inactive, whereas a sand with water-softening powers and thus presumably containing some zeolite was highly active even after ignition. Cowie's results were confirmed under Indian conditions (8*a*).

Cowie's work was followed up in these laboratories by A. G. Pollard in unpublished experiments on a number of minerals, some of which may occur in the coarser fractions of the soil. The materials were added to quartz sand with sufficient water to maintain a crumbly state and treated with free cyanamide and "Nitrolim" (an early form of unoiled dusty Calcium Cyanamide) at the rate of 80 mg. N per kg. of mixture. The data in Table I give the results for the most active zeolite, prehnite, at four rates of admixture with sand and at four intervals, for experiments with free cyanamide. For the other minerals the table gives the proportion of nitrogen found as urea after 20 days in mixtures containing 8 per cent. of the mineral. Only two of the materials tested, prehnite and apophyllite, produced urea from Calcium Cyanamide. Both of these zeolites contain hydrogen other than water, and it is probable that they took up the excess lime in the Calcium Cyanamide and reduced the alkalinity. No cyanamide remained and no ammonia was formed in the experiment with prehnite. In the other tests the usual polymerisation to dicyanodiamide probably occurred in the alkaline solutions formed from the commercial fertiliser. Six other minerals caused urea formation from free cyanamide and a large number proved inactive. These included four zeolites or associated minerals, three of which had given positive results in Ulpiani's tests. It is not possible from these experiments to relate catalytic power to chemical composition, but it is clear that the property is so widespread that cultivated soils are rarely likely to be devoid of the necessary catalyst for cyanamide decomposition.

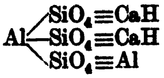
Pollard also tested the catalytic power of a number of soils using Cowie's proposal for preventing nitrification by adding dicyanodiamide. This makes it possible to measure the decomposition by determining together the urea and ammonia formed. The data in Table II give the time required for the maximum conversion of cyanamide to urea or ammonia and the percentage of the added nitrogen so transformed. In no case was the conversion complete under the conditions of these experiments, though in several soils it reached 90 per cent. Only a bog soil was relatively inactive; two fen soils both with high organic matter contents were highly active, thus differing completely from the fen soil studied by Cowie. In the light loam and light sand the decomposition of unoiled Calcium Cyanamide proceeded more rapidly than that of free cyanamide, but in the other soils, as with the minerals, the free cyanamide was decomposed more rapidly. It will be noted that the Woburn soil, which is derived from Lower Greensand material and contains glauconite, retained its power of urea formation even after ignition. This soil was

Table I. *Formation of urea from free cyanamide and Calcium Cyanamide in moist sand (A. G. Pollard's data).*

GROUP I.

Urea formation from both free cyanamide and Calcium Cyanamide.

(1) Prehnite*†

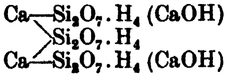


Percentage urea from free cyanamide.

% prehnite in mixture	Time in days			
	1	4	8	21
1	—	—	2	25
5	10	30	40	90
10	20	55	70	90
25	30	100	100	100

Percentage urea from Calcium Cyanamide.

(1) Prehnite*† ... 22 %
(2) Apophyllite*† ... 17 %



GROUP II.

Urea formation from free cyanamide but not from Calcium Cyanamide.

			% urea formed
(3)	Orthoclase	$KAlSi_3O_8$	55
(4)	Albite	$NaAlSi_3O_8$	45
(5)	Wavellite	$2Al_3(OH)_3(PO_4)_2.9H_2O$	45
(6)	Phillipsite*	$R\ Al_2Si_4O_{12}.4H_2O$ (R chiefly Ca)	40
(7)	Thomsonite*	$2(CaNa_2)O.2Al_2O_3.4SiO_2.5H_2O$	15
(8)	Glauconite	$FeK(SiO_3)_2.nH_2O$	10

GROUP III.

No urea formation from either substance.

(9)	Natrolite*†	$Na_2Al_2Si_3O_{10}.2H_2O$
(10)	Chabazite*†	$CaAl_2(SiO_3)_4.6H_2O$
(11)	Analcite*†	$NaAl(SiO_3)_2.H_2O$
(12)	Scolecite*	$CaO.Al_2O_3.3SiO_2$ 3 [*i.e* $3H_2O$] H_2O
(13)	Stilbite†	$(CaNa_2)Al_2(Si_3O_8)$ 2 [*i.e.* $(Si_3O_8)_2$] $6H_2O$
(14)	Pectolite	$4CaO.Na_2O.6SiO_2.H_2O$
(15)	Mica	$H_2KAl_3(SiO_4)_3$
(16)	Talc	$H_2Mg_3(SiO_3)_4$
(17)	Serpentine	$H_4(MgFe)_3.Si_2O_9$
(18)	Apatite	$3Ca_3(PO_4)_2.CaF_2$
(19)	Hornblende	
(20)	Precipitated silica	
(21)	Dialysed silica	
(22)	Precipitated calcium silicate	
(23)	Ignited ferric oxide	

* Zeolitic or related substance.
† Positive results in Ulpiani's experiments.

Table II. *Urea and ammonia formation in soils* (*A. G. Pollard's data*).

(Additions at the rate of 96 mg. N per kg. soil.)

				From free cyanamide		From Calcium Cyanamide (unoiled dusty nitrolim)	
	Description	pH	Temp.	% N as urea + ammonia	Time in days	% N as urea + ammonia	Time in days
Rothamsted	Heavy loam	>7	Room	40	3	60	10–15
Rothamsted	Heavy loam	>7	30°	70	3	90	5– 6
Redbourn	Loam	5·5	Room	50	2	55	10–15
Woburn	Light loam	6·5	Room	50	30	50	10–15
Woburn	Light loam	—	30°	70	4– 5	90	4– 5
Blackheath	Light sand	4·0	25°	70	12–15	90	10
Methwold	Fen	—	25°	70	4– 5	90	4– 5
Fen	Fen	6·5	25°	—	—	70	6
Entwistle	Bog	—	Room	—	—	10	14
Ignited Rothamsted soil		>9	Room	—	—	Nil	—
Ignited Woburn soil		—	Room	—	—	12	5

acid and contained little replaceable calcium and the ignited material was therefore not strongly alkaline. The Rothamsted soil contained calcium carbonate and much replaceable calcium and became strongly alkaline on ignition. The absence of urea formation in the ignited Rothamsted soil affords further evidence that the formation of urea from Calcium Cyanamide requires not only a suitable catalyst but the removal of the excess lime contained in the fertiliser. As has already been shown, high alkalinity favours the formation of dicyanodiamide instead of urea.

THE TOXICITY OF CYANAMIDE AND ITS PRODUCTS TO SEEDS AS RELATED TO ITS DISAPPEARANCE FROM SOIL.

A more detailed series of measurements on the decomposition of cyanamide in soils under controlled conditions of moisture and temperature was carried out by the late A. J. Walker in the course of an examination of the effect of Calcium Cyanamide and its constituents on the germination of seeds. The results of field experiments on germination will be given in a later paper in a general discussion on the agricultural use of Calcium Cyanamide. The laboratory results proved to be so closely connected with the decomposition of the cyanamide that they are treated here.

When Calcium Cyanamide is mixed with moist soil a variety of substances is produced (*e.g.* acid calcium cyanamide, free cyanamide, calcium hydroxide, urea, ammonia, both free and combined, and traces of acetylene and phosphine). Each of these has at some time been held responsible for the injurious effect on germination often observed after the injudicious use of Calcium Cyanamide in practice. At concentrations equivalent to those produced from heavy Calcium Cyanamide dressings neither slaked lime nor urea had any appreciable effect on germination in moist soil under laboratory conditions. The absence of any effect from urea, which is rapidly ammonified, makes it improbable that ammonia is a source of injury during the decomposition of Calcium Cyanamide, except possibly where the material is applied very irregularly and the ammonia is produced near soil which is still highly alkaline from the calcium hydroxide formed from the calcium oxide and calcium cyanamide. The gaseous products from the decomposition had no perceptible effect on seeds germinating on filter paper over moist soil and Calcium Cyanamide in closed vessels.

On the other hand, seeds moistened on filter paper with a solution of free cyanamide or with an extract of Calcium Cyanamide were killed before germination occurred. Undecomposed cyanamide can penetrate the seed coat and kill the seed even before the embryo emerges. It is uncertain whether free cyanamide or the acid salt is the agent responsible in the soil, but this point seems of little importance. Further support for the direct toxicity of cyanamide is afforded by the experiments described in the following section on the relationship between the toxicity and the conditions under which the Calcium Cyanamide is added to the soil.

THE RATE OF DISAPPEARANCE OF CYANAMIDE FROM THE SOIL.

Our own and earlier field experiments showed that Calcium Cyanamide applied in the field and cultivated into the soil a week or so before sowing the seeds had little or no toxic action. With shorter intervals the results varied with the weather conditions; even applications with the seed were occasionally innocuous. The effects of the moisture content and the temperature of the soil on the rate of disappearance of cyanamide and on the germination of seeds were therefore examined under controlled conditions in the laboratory. A heavy dressing corresponding to about 1 ton of Calcium Cyanamide per acre to 9 in. (215 mg. N per kg. dry soil) was used to compensate for the greater intimacy of incorporation with the soil in the laboratory tests and to facilitate the analyses. The tests were conducted in Rothamsted heavy loam soil at five moisture contents (5, 11, 14·5, 19 and 25 per cent.) and at two temperatures (13·5° and 22·5°), for applications of Calcium Cyanamide or equivalent amounts of free cyanamide. Samples of soil were withdrawn for deter-

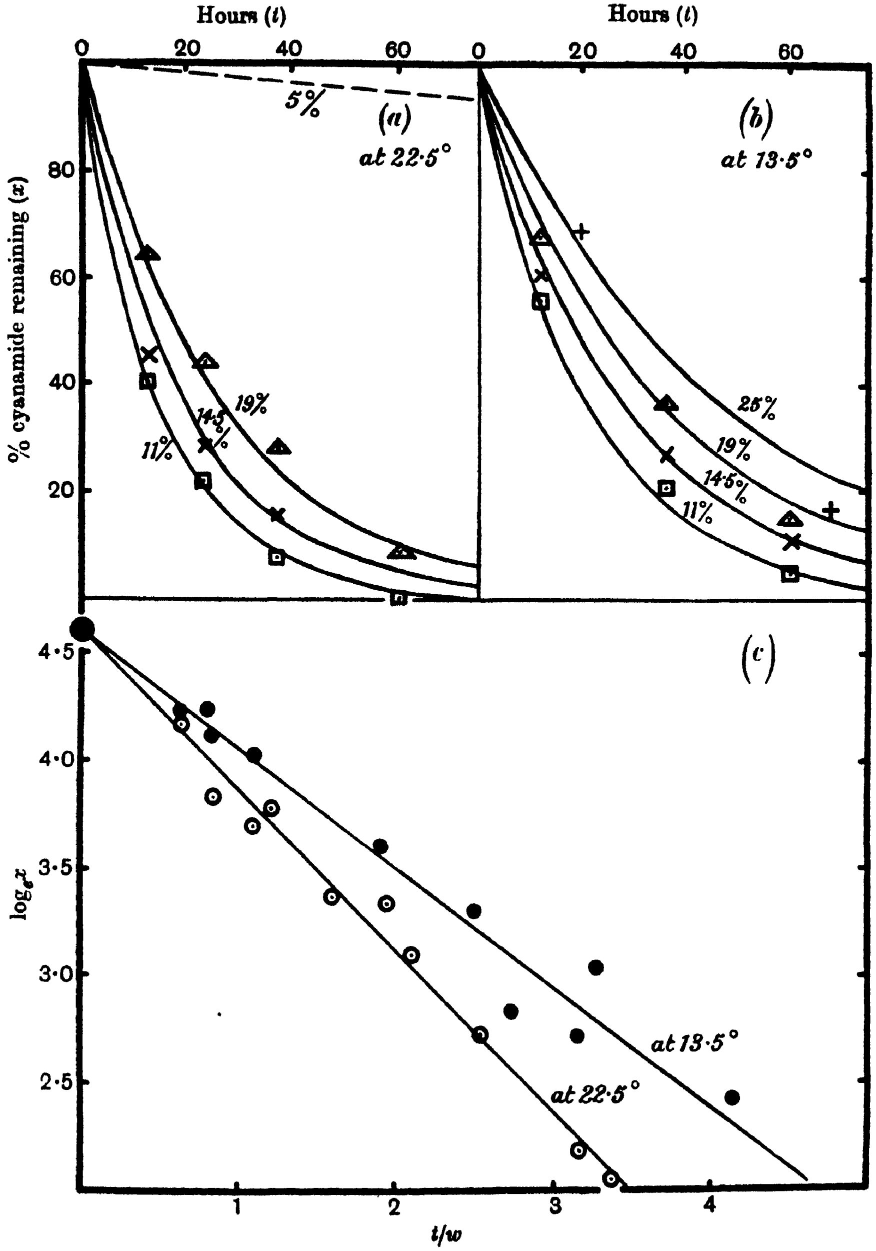


Fig. 1. Rate of disappearance of Calcium Cyanamide from Rothamsted soil under laboratory conditions. (*a*) and (*b*): percentage of added cyanamide (x) remaining after t hours in soils of moisture contents (w) of 25, 19, 14·5, and 11 per cent., at 22·5° and 13·5° respectively. Dotted line for 5 per cent. moisture. (*c*): the same data with $\log_e x$ plotted against t/w. (A. J. Walker's data.)

minations of the amount of cyanamide remaining, and on precisely similar lots of soils seeds, known from field experiments to be of highly susceptible types, were sown at intervals of 0, 12, 36 and 60 hours after adding the cyanamide.

The points in Figs. 1 *a* and 1 *b* show the amounts of cyanamide found by analysis plotted against the time after incorporating the cyanamide.

Over the range of 11–25 per cent. of moisture and of 13·5–22·5° the disappearance of cyanamide proceeded more rapidly at the higher temperature and in the drier soils. With 19 per cent. of water in soil at 22·5° the disappearance was about as rapid as with 14·5 per cent. of water at 13·5°. In very dry soil (5 per cent. moisture) the decomposition was very slow. Free cyanamide disappeared much more rapidly than cyanamide added in the commercial form, presumably because it was added in solution and so came more rapidly into contact with the soil colloids.

In Fig. 2 the natural logarithms of the cyanamide contents of the soil are plotted against the ratio of the time to the moisture content of the soil. Within the limits of the analyses the data for three moisture contents at each temperature fall satisfactorily on the straight lines:

$$\log_e \frac{100}{x} = M.\frac{t}{w} \quad \text{or} \quad x = 100e^{-\frac{mt}{w}},$$

where x is the percentage of the added cyanamide remaining after t hours in soil of moisture content w and m has the values 0·75 at 22·5° and 0·54 at 13·5°. The full-line curves in Fig. 1 also represent this general equation.

The relative disappearance rate of cyanamide is thus inversely proportional to the moisture content, and the actual rate of disappearance is proportional to the concentration of cyanamide in the soil solution

$$-\frac{1}{x}\frac{dx}{dt} = \frac{m}{w}.$$

This simple logarithmic curve suggests a unimolecular homogeneous reaction, but the reaction undoubtedly proceeds according to the second order equation

$$HCN_2 + H_2O \rightarrow CO(NH_2)_2.$$

This would, however, behave as a pseudo-unimolecular reaction, since the solutions are so dilute that the concentration of one reactant (water) is greatly in excess of the other. The formation of urea from cyanamide in soil is probably a heterogeneous reaction catalysed at a solid surface, and the above simple logarithmic equation is equally in accordance with this catalysis under the condition that the amount of active surface covered at any time by cyanamide is proportional to the concentration of cyanamide in the soil solution. Further, the temperature coefficient of the change is at the rate of 1·4 per 10° temperature rise and this is in harmony with a reaction dominated by a diffusion process rather than by a chemical change. Although the above simple equation for the rate of change cannot be used as evidence on the mechanism of the reaction, it serves as a useful interpolation formula for calculating the cyanamide concentration at any time or the mean value over any interval. Such calculations are used in the following section.

THE EFFECT OF MOISTURE CONTENT AND TEMPERATURE ON THE TOXICITY OF CYANAMIDE TO SEEDS.

Fig. 2 gives typical curves for the rate of germination of seeds under laboratory conditions with applications of Calcium Cyanamide to moist soil at the time of sowing the seeds. The cyanamide not only reduced the total germination but so modified the whole course of the germination curve that it became a matter of some difficulty to compare results for different environmental conditions and treatments. The final germination alone was unsatisfactory, for some treatments allowed germination to proceed, slowly, long after all the untreated seeds had germinated. The time for the germination of one-quarter of the seeds sown was therefore taken as a combined measure of retardation and reduction of germination. It has the advantage of being applicable to all treatments and of falling on the steepest part of the germination curves and so reducing errors of graphical interpolation.

The results for all experiments with moisture contents comparable with field conditions are shown in Fig. 3.

The interval between adding the cyanamide and sowing is clearly of great importance. Even a 12-hour interval greatly reduced the injurious effect; 36 hours reduced it still more, and with a 60-hour interval the effect was practically negligible at the higher temperature. The mean concentrations throughout the first 24 hours after sowing the seeds were calculated from the logarithmic equations discussed for the appropriate periods after the addition of the cyanamide (*i.e.* 0–24, 12–36, 36–60, 60–84 hours), and in Fig. 3 these mean concentrations are plotted against the times for 25 per cent. germination. It will be seen that they fall sufficiently closely to straight lines to justify the conclusion that at each temperature the retarding effect of Calcium Cyanamide upon germination depends on the amount present shortly after the seeds are sown, and that this in turn depends not only on the actual amount of cyanamide present but also on the rate at which it is disappearing.

At the lower temperature germination was much slower and less complete and the cyanamide caused greater actual retardation and final injury. Within the range studied, temperature had a much greater effect than moisture content on the actual toxicity to germination. The retardation relative to untreated seed for a given cyanamide concentration was much the same at the two temperatures.

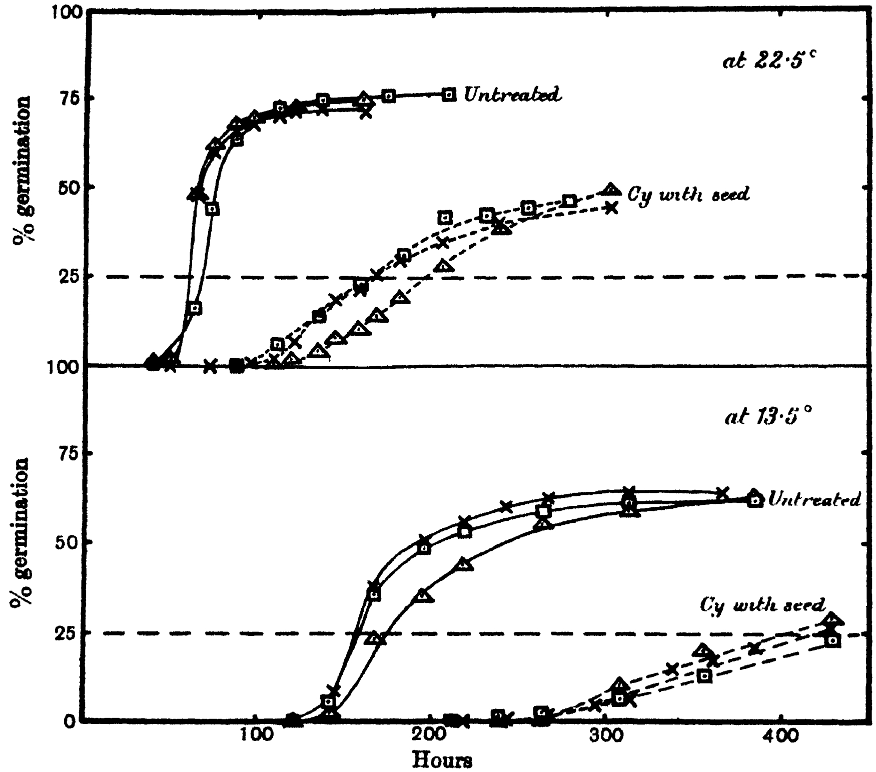


Fig. 2. Rate of germination of swedes under laboratory conditions at 13·5° and 22·5° in soil with and without Calcium Cyanamide added at time of sowing. Moisture contents of soils: triangles 19 per cent.; crosses 14·5 per cent.; squares 11 per cent. Rothamsted soil with cyanamide N at the rate of 215 mg. per kg. soil. (A. J. Walker's data.)

The fact that the actual toxic effect should be so much less for germination at the higher temperature is contrary to a view sometimes put forward that the embryo is protected within the seed but passes through a sensitive stage as it emerges. If this were true it would be expected that the toxicity should be increased at higher temperatures, for with more rapid germination the embryo should be exposed whilst appreciable amounts of cyanamide still remained. On the other hand,

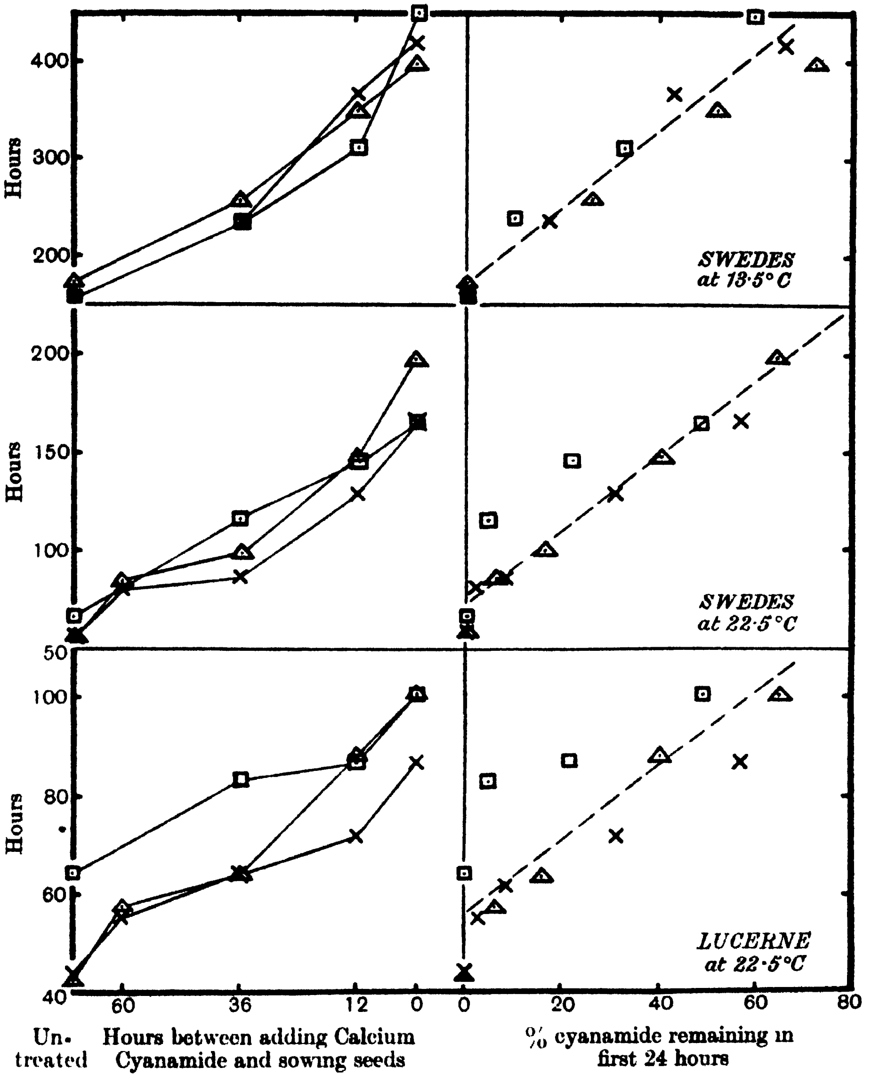


Fig. 3. Relationship between times required for germination of 25 per cent. of seeds sown and (1) left—interval in hours between addition of Calcium Cyanamide and sowing of seeds, (2) right—calculated mean cyanamide content of soil during first 24 hours after sowing seeds. Moisture contents of soils; triangles 19 per cent.; crosses 14·5 per cent.; squares 11 per cent. (A. J. Walker's data.)

the greater toxicity at the lower temperature is in harmony with the relationship between toxicity and mean cyanamide concentration during the first day, and again with the observations on the rapid killing of seeds by cyanamide solutions. The toxic action is exerted within the seed as soon as soil solutions containing cyanamide are imbibed by the seed. Presumably cyanamide so absorbed decomposes less quickly than that remaining outside in contact with soil colloids, and the toxic action may therefore be exerted for longer periods and act continuously until either the seed dies or the concentration is reduced below the toxic limit by the further imbibition of water with a cyanamide content which is rapidly falling. Conditions favourable to rapid germination will therefore reduce the risk of damage both by this direct effect and also indirectly, for conditions favourable for good germination will also favour the mechanical distribution and diffusion of cyanamide and thus its rapid decomposition.

Since the concentration used in these laboratory tests was ten to twentyfold that normally used in the field it seems likely that, in practice, the 7–10 days' interval recommended for safety might be reduced, provided that the conditions are suitable for the rapid conversion of cyanamide to urea. In part these depend on uncontrollable factors such as the soil type and the weather. Differences between soils are well shown in our pot experiments; the greater part of the cyanamide added disappeared in 1 day in a fen soil, in 2 or 3 days in a heavy loam soil, but required a week in an acid light loam. In any case the essential factor is the intimate incorporation of the cyanamide with the soil. This requires not only the careful choice of weather conditions but an efficient harrowing or other form of cultivation between applying the fertiliser and sowing the seed. The efficiency of this cultivation is more important than the mere length of the interval. The moisture content is of less practical importance provided extremes are avoided. In drier soils the rate of decomposition is more rapid but diffusion is slower. Extreme dryness or wetness should be avoided, as they impede efficient distribution both mechanically and by diffusion. Cyanamide left on the surface of a dry soil may retain its toxicity for many days until rain washes it down to the soil and seeds. Great irregularity of distribution or extreme dryness or wetness may have adverse effects in another direction, for the carbon dioxide content of the soil air and water may then be insufficient to convert the calcium hydroxide rapidly into calcium bicarbonate and the decomposition may thus proceed in an alkaline solution with the formation of dicyanodiamide. This material is probably not as toxic as cyanamide, but it is so much more stable that its effect is exerted over a much longer interval of time and the ultimate effect may be as serious.

THE FORMATION OF AMMONIA AND NITRATE FROM CYANAMIDE.

The final stages in the decomposition of Calcium Cyanamide in the soil which bear more directly on the nutrition of the plant are effected by the soil micro-organisms. For these reasons the experiments to be discussed in the present section were planned in conjunction with pot cultures or field experiments or with studies on the soil microflora. The biological aspects will be discussed more fully in the following papers; here it is convenient to bring together the chemical data so as to bring out the influence of cyanamide on the accumulation of ammonia and nitrate in different kinds of soil and under different environmental conditions.

Particular attention should be given in experiments on cyanamide and similar products to the rate of application. In much of the published work the additions were a hundred times as heavy as is ever likely to be desired in practice. In the present work an attempt was made to find a better compromise between normal field dressings and those required to give reasonable accuracy in the analytical work. Owing to inevitable irregularities of distribution of fertiliser in the field, applications of several times the normal rate will be common, but it is now known that the toxicity and disturbing influence of cyanamide and its products increase so rapidly with concentration that laboratory studies on tenfold or hundredfold dressings can have little bearing on the field behaviour. In our pot experiments the dressings were several times normal field rates.

In interpreting the behaviour of Calcium Cyanamide in the field it is necessary to know the relative duration of the successive stages. Thus, leaching is important for the period in which the cyanamide remains as the soluble acid salt or as urea in the soil, for there is no evidence that these are absorbed to any appreciable extent, whereas during the period of ammonia accumulation risk of loss by leaching is very greatly reduced, as the ammonia is firmly held by the clay in the exchangeable form. A retardation of nitrification may therefore reduce nitrate losses by leaching, by postponing the time of rapid nitrate formation to a period when the growing crop is able to absorb nitrate rapidly.

The experiments were conducted on the following soils:

(1) A very acid loam on the Millstone Grit at Stalybridge, Cheshire. This soil received calcium carbonate to give pH values of about 6.

(2) A neutral or slightly acid sandy loam from Lower Greensand at Woburn, Beds.

(3) A heavy loam with calcium carbonate from Clay-with-Flints at Rothamsted, Herts., taken from land deficient in nitrogen.

(4) A highly calcareous soil from Drift on Chalk at Leagrave, Beds.

(5) A calcareous fen soil from Cambridgeshire rich in nitrogen and showing little response to nitrogen in pot experiments.

In 1927 experiments on the decomposition of Calcium Cyanamide, free cyanamide, urea and ammonium sulphate were conducted by A. J. Walker in Millstone Grit, Rothamsted, and fen soils in a series of uncropped pots running parallel with a series of pots cropped with barley but taken down, thoroughly remixed, and sampled at frequent intervals for determinations of nitrogen as cyanamide, dicyanodiamide, urea, ammonia and nitrate. Heavy dressings (75, 80 and 150 mg. N per kg. of dry soil respectively) were used to facilitate the analyses. The results are given graphically in Fig. 4 and summarised in Table III. The outstanding feature is the rapidity of the first two changes to urea and ammonia. Both urea and cyanamide disappeared within 3 or 4 days, and even after 14 hours less than one-tenth of the cyanamide nitrogen was found as urea. It is well known that urea is ammonified extremely rapidly in normal soils, and urea produced from Calcium Cyanamide or free cyanamide behaves as urea added directly. Presumably the ammonification is brought about by micro-organisms and not by a free urease.

The accumulation of ammonia from cyanamide or urea was so rapid that within 5 days the ammonia contents were almost identical for all sources of nitrogen. The amount of ammonia accumulated depended, of course, on the rapidity with which it was removed again by nitrification. In the active fen soil the ammonium content when the curves converged was only one-half of the nitrogen added and in the much less active Cheshire soil the ammonia accumulation approached the theoretical maximum for full conversion. After a period of 3–10 days depending on the general biological activity of the soil the ammonia and the nitrate curves began to show wide divergences according to the source of the ammonia. Where cyanamide had been added in either form, oxidation of the ammonia proceeded much more slowly than with ammonium sulphate or urea even though both cyanamide and urea had disappeared completely from the soil.

The rapidity of the conversion of cyanamide nitrogen to ammonia and the slowness of the subsequent changes bring out a point of the utmost importance in interpreting the behaviour of Calcium Cyanamide as a fertiliser. It has commonly been believed that nitrification in arable soils proceeds so rapidly that the accumulation of nitrate nitrogen

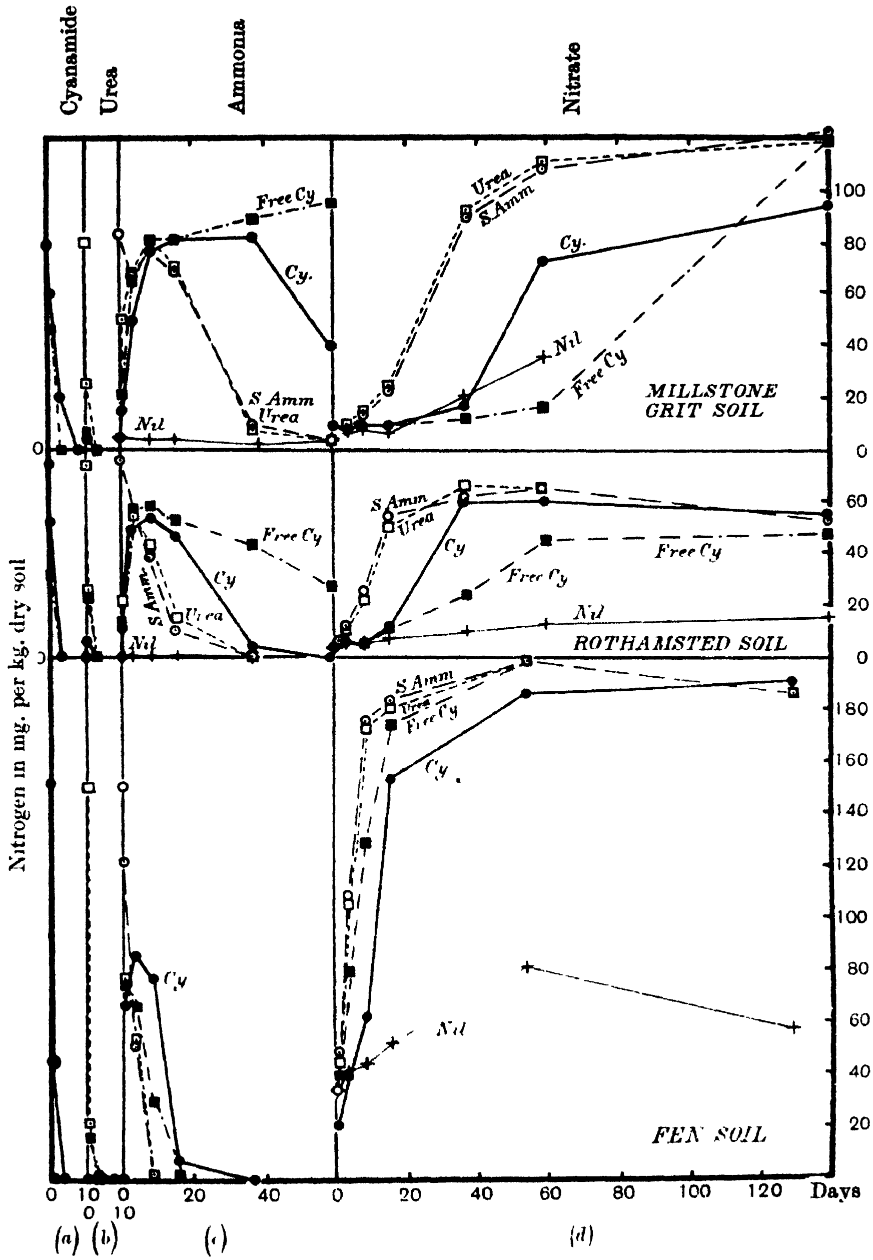


Fig. 4. Decomposition of Calcium Cyanamide (full circles), free cyanamide (full squares), urea (open squares) and ammonium sulphate (open circles) in three soils (Millstone Grit, Rothamsted and fen). The curves show on a uniform time scale the amounts of N in mg. per kg. soil as (*a*) cyanamide, (*b*) urea, (*c*) ammonia, (*d*) nitrate. (A. J. Walker's data.)

measures the production of ammonia rather than the time required for the actual oxidation to nitrate. The slower action of Calcium Cyanamide has sometimes been explained by the time required for the long series of chemical and biological processes which must precede the final oxidation. The data presented in this paper show that this view is totally wrong. *Under normal soil conditions the preliminary stages in the decomposition of cyanamide proceed very rapidly indeed, and the slowest stage is the final oxidation or nitrification.*

The rate of oxidation of ammonium sulphate varied from soil to soil, but in all cases ammonia from cyanamide was oxidised much more slowly than that from ammonium sulphate. In the extreme case (Millstone Grit soil) no nitrate was formed from Calcium Cyanamide during the first 6 weeks, by which time oxidation of ammonia from ammonium sulphate or urea was almost complete; the delay was even greater for free cyanamide. There was about a fortnight's delay in the Rothamsted soil, but less than a week's delay in the fen soil.

The duration of the inhibition period cannot be determined with sufficient accuracy from the commencement or completion of nitrification since the curves are roughly sigmoid. The point for the accumulation of nitrate equivalent to one-half of added nitrogen in excess of the untreated soil was therefore used as the best measure of the course of the change. Values obtained graphically are given in Table III. (For field comparison nitrate contents are rendered less suitable by unknown losses through leaching and the times for the reduction of the ammonia contents to one-half of the initial value for ammonium sulphate may be used instead.) Cyanamide nitrogen disappeared so rapidly that simple graphical interpolation was not possible. By assuming that the disappearance followed the simple logarithmic course already discussed, the periods for half disappearance may be obtained from the initial values and those after 14 or 16 hours. The rates of the principal changes in the three soils are given in Table III in terms of these half periods in units of days and also relative to the half periods for the least active soil.

As in the laboratory experiments, free cyanamide added in solution disappeared about twice as quickly as that added in solid Calcium Cyanamide. For both materials the Rothamsted soil was about twice as active as the Millstone Grit soil, and the fen soil about three times as active as the Rothamsted soil. The formation of nitrate from Calcium Cyanamide required about two and a half times as long in each of the soils as that from ammonium sulphate or urea. Although one-half of the cyanamide from Calcium Cyanamide disappeared in from 9 to

42 hours, it required about 33 times as long before this amount of nitrogen was converted into nitrate.

Table III. *Comparison of rates of nitrate formation and disappearance of cyanamide in three soils by times for completion of one-half of the changes* (*A. J. Walker's data*).

	Half periods in days			Relative values, Millstone Grit = 100		
	Mill-stone Grit soil	Rotham-sted soil	Fen soil	Mill-stone Grit soil	Rotham-sted soil	Fen soil
Disappearance of cyanamide from:						
Calcium Cyanamide	1·75	1·13	0·38	100	64	22
Free cyanamide	0·84	0·49	(0·2)	100	58	(20)
Accumulation of nitrate from:						
Ammonium sulphate	25	13	4·2	100	52	17
Urea	24	14	4·6	100	58	19
Calcium Cyanamide	62	31	13·5	100	50	22
Free cyanamide	(100)	66	7·5	—	—	—
Lag in nitrification:						
Calcium Cyanamide: ammonium sulphate	37	18	9·3	100	49	25
Ratio of periods for half nitrification:					Mean for 3 soils	
Calcium Cyanamide: urea	2·6	2·2	2·9		2·6	
Calcium Cyanamide: ammonium sulphate	2·5	2·4	3·2		2·7	
Ratio of period for half nitrification to period for half disappearance						
Calcium Cyanamide	35	28	35		33	
Dicyanodiamide nitrogen as per cent. of N added from:						
Calcium Cyanamide after 8 days	5·8	1·3	0·0			
,, 54 days	2·6	0·8	0·3			
Free cyanamide after 8 days	5·7	1·9	0·3			
,, 54 days	4·0	0·6	0·3			

Free cyanamide caused a greater retardation of nitrification than Calcium Cyanamide in the Millstone Grit and the Rothamsted soils but not in the fen soil, even though it disappeared more rapidly in all three soils. The high initial concentration of cyanamide from a solution of free cyanamide would be expected to prove more toxic than the lower concentrations produced more slowly by the hydrolysis of Calcium Cyanamide; in the fen soil which effected all the changes with extreme rapidity the effect of this high concentration was apparently outweighed by the more rapid disappearance of the free cyanamide.

The relative efficiencies of the three soils were surprisingly similar for all the changes considered, the average of the relative values for the several half periods given in Table III being 100 for the Millstone Grit soil, 55 for the Rothamsted soil and 21 for the fen soil. Some general agreement between the duration of cyanamide in the soil and the lag in nitrification would be anticipated, although the relationship found was unexpectedly close. It is not, however, so easy to understand why the rate of disappearance of cyanamide, which is essentially the work of an inorganic catalyst, should be so closely parallel to the microbiological activity of the soil as measured by the rate of oxidation of ammonium sulphate. It must be remembered, however, that the three soils differed widely in physical and chemical properties as well as in their micro-floras. In the Rothamsted loam and still more in the highly organic fen soil dissolved materials would be brought into more intimate contact with the soil colloids than in the light Millstone Grit soil. Further, since both the buffer capacity of the colloids and the carbon dioxide production in the early stages of the experiment would increase in the order (1) Millstone Grit, (2) Rothamsted, (3) fen, the rate of neutralisation of the alkalinity of the Calcium Cyanamide would be in this order. Some evidence that side reactions were greatest in the Millstone Grit soils was given by the dicyanodiamide determinations included in Table III. Although no great precision is claimed for these results, they show in the extent of the side reactions in cyanamide decomposition the soils were in the above order, *i.e.* greatest where the alkaline material remained longest. Although traces of dicyanodiamide were detected throughout the experiment, it is improbable that this substance was ever present in sufficient amount to be responsible for the retarded nitrification. The toxic action is so closely related to the disappearance of the cyanamide that here, as in the germination experiments, it is sufficient in the light of our present knowledge of cyanamide decomposition to regard the cyanamide itself as the toxic agent. Whatever the mechanism, these experiments agree with those on germination in showing that the risk of disturbance from Calcium Cyanamide is least in fertile soils in good condition for crop growth. In general, adverse conditions of whatever nature emphasise the differences between the behaviour of Calcium Cyanamide and of the simpler forms of nitrogen.

A second detailed series of comparisons of ammonium sulphate and Calcium Cyanamide in the Millstone Grit, Woburn, Rothamsted and Leagrave soils was made in 1928 by H. L. Richardson. The conditions resembled those in cropped pots or in the field still more closely for the soil was not disturbed for sampling and the rates of application (40 mg. N per kg.) were lower. Long porous tubes were inserted down the centre of the pots which were cropped with barley, and samples of the soil solution were drawn off by suction from time to time for nitrate determinations. The moisture contents of the soils were adjusted as closely as possible to constant values, and the points for independent duplicate pots in Fig. 5 show satisfactory agreement. The nitrate contents give the excess of nitrate production over its absorption by the plants and soil micro-organisms, but in the early stages the effect of the plant was small, as was shown in the nitrate contents of the untreated soils. The results for the first 5 or 6 weeks may be regarded as measuring total nitrate production and are therefore presented here for comparison with the earlier experiment. The relationships between nitrate contents and the growth of the crops will be discussed in a later paper.

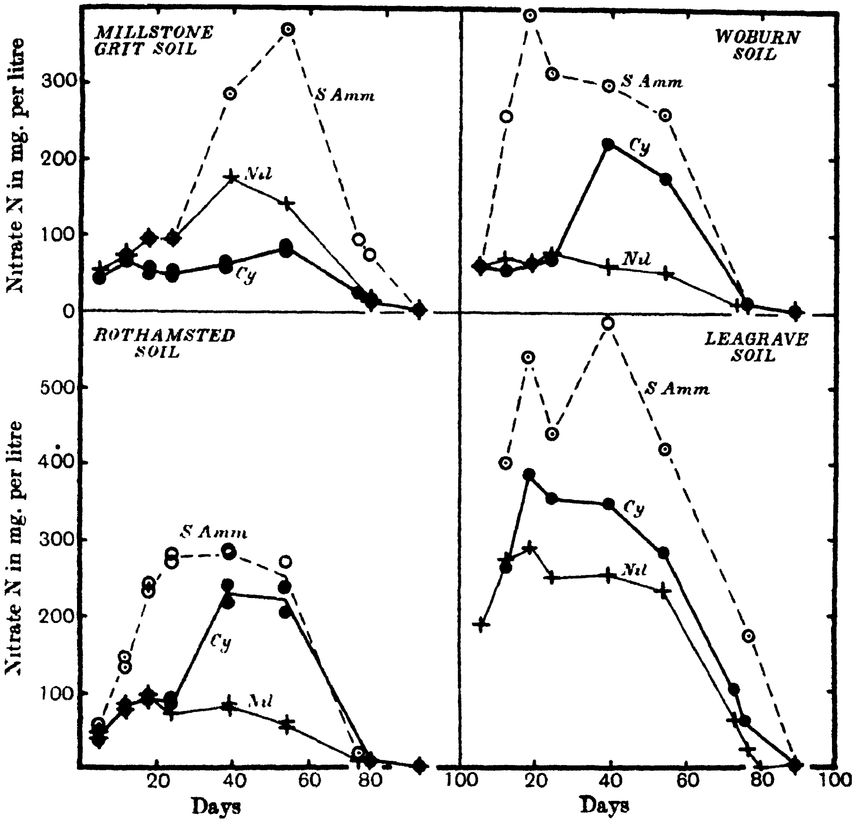


Fig. 5. Nitrate contents in mg. N per litre of soil solution extracted by suction through porous cylinders from cropped pots of four soils treated with ammonium sulphate or Calcium Cyanamide or without added nitrogen. Duplicate pots shown for Rothamsted soil and for Millstone Grit soil with Calcium Cyanamide. (H. L. Richardson's data.)

The Millstone Grit soil again nitrified more slowly than the other soils. There was no accumulation of nitrate from ammonium sulphate for 3 weeks and none at all from Calcium Cyanamide. Throughout the 13 weeks in which nitrate determinations were made there was much less nitrate from Calcium Cyanamide than from the untreated soil. (It may be mentioned that this interference with nitrification had no adverse effect on the crop which at some stages appeared even better than the one with ammonium sulphate.)

In the Rothamsted and Woburn soil nitrate formation from Calcium Cyanamide lagged nearly 3 weeks behind that from ammonium sulphate. The Leagrave soil proved to be very rich in both nitrate and readily nitrifiable material. The delay from Calcium Cyanamide was less prolonged, but throughout the experiment the extra nitrate from Calcium Cyanamide was only from one-third to one-half of that from ammonium ulphate.

In the two soils common to the two experiments nitrification, whether from ammonium sulphate or from Calcium Cyanamide, was appreciably slower in the undisturbed cropped pots than in the uncropped pots mixed and well aerated during sampling.

THE INFLUENCE OF AERATION ON NITRIFICATION IN POT EXPERIMENTS.

The experiments in pots in 1927 and 1928 suggested a reduction in microbiological activity through inadequate aeration in the customary form of deep glazed earthenware pots used. We have had much other evidence of the impeded aeration in pot cultures, and in 1929 experiments conducted by B. K. Mukerji gave a further illustration of the effect of different degrees of aeration on the decomposition of ammonium sulphate and Calcium Cyanamide. A number of uncropped pots of Rothamsted soil without nitrogen and with ammonium sulphate or Calcium Cyanamide were set up in the early winter for the microbiological studies described more fully in the second paper of this series (9). For each treatment a new undisturbed pot was sampled by means of an auger every fifth day, and the samplings were repeated daily for 5 further days. In this way a series of comparisons was provided for completely undisturbed soil against pots which had been slightly disturbed and aerated on the 5 preceding days by taking out a small cylinder of soil. As the experiment was continued for 50 days many of the soils were aerated for only a small fraction of the total period before sampling. The extra aeration was, however, sufficient to cause appreciable differences in the rates of disappearance of ammonia and formation of nitrate and, in particular, in the relative effects of ammonium sulphate and Calcium Cyanamide. The results for the undisturbed and the aerated series are given in Fig. 6. It will be seen that the disappearance of ammonia from ammonium sulphate and both the accumulation and disappearance of ammonia from Calcium Cyanamide proceeded more slowly in the undisturbed pots. There was little loss of ammonia from ammonium sulphate for the first 3 weeks in the undisturbed soil, whereas half of the ammonia had gone by this time from the aerated series. The maximum ammonia accumulation from cyanamide was only about one-half of the theoretical amount in the undisturbed series, but it was almost complete in the aerated series. The differences in nitrate production were not so pronounced as in ammonia loss, but nitrification proceeded more smoothly in the aerated series and there was a more pronounced period of inhibition in the undisturbed series.

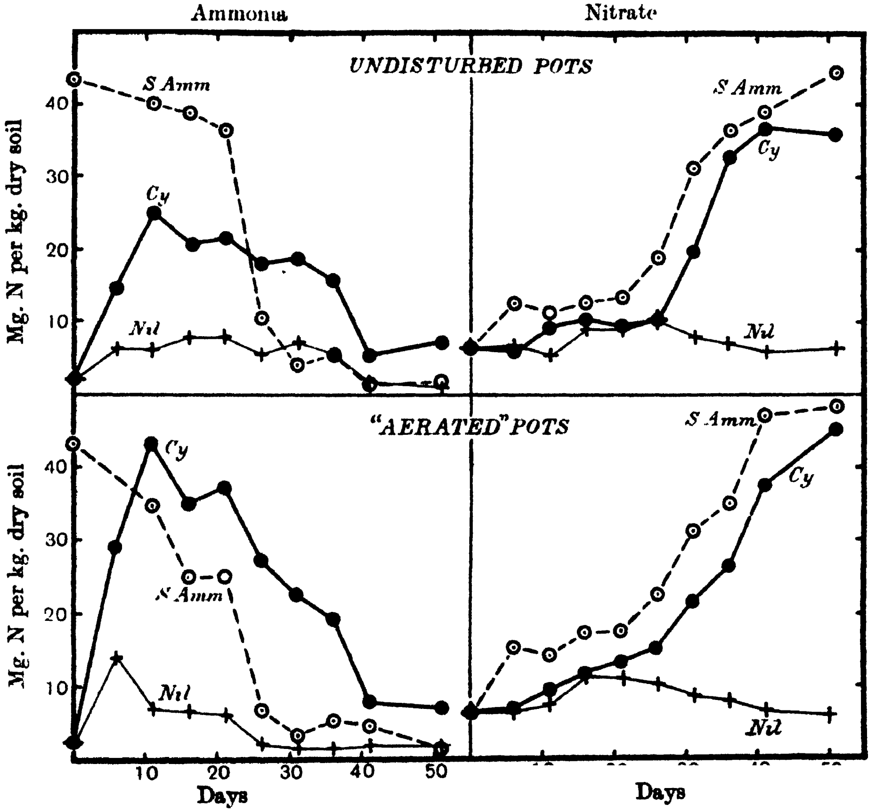


Fig. 6. Production of ammonia and nitrate from Calcium Cyanamide and ammonium sulphate in uncropped pots. Upper half—undisturbed pots; lower half—pots disturbed and aerated by soil sampling on 4 preceding days. (B. K. Mukerji's data.)

It will be noted that although the nitrification of ammonium sulphate was slower in these experiments than in the 1927 and 1928 experiments, the lag caused by Calcium Cyanamide was less. The slower nitrification was undoubtedly caused by the lower temperature of the early winter months, but the cause of the reduction in the cyanamide lag is less clear. It may be that storage in an air-dry condition in a soil bin throughout the summer led to spore formation and made the organisms more resistant to cyanamide.

THE NITRIFICATION OF CALCIUM CYANAMIDE UNDER FIELD CONDITIONS.

Field studies on nitrate formation and accumulation are necessarily less precise than those conducted in pot or laboratory cultures. In addition to inevitable irregularities in the distribution and incorporation of fertilisers and the errors of soil sampling, uncontrolled and highly variable displacements of the soil solution and leaching out of soluble salts prevent any detailed study of the changes occurring. General indications may, however, be obtained from the earlier stages of field experiments, especially if the ammonia content is followed in addition to the nitrate content. Experiments were therefore made to ascertain how far the field behaviour resembles that in pots, for it has been shown that the pot experiments already considered differed from field trials in such important particulars as the regularity of distribution and incorporation, the degree of aeration and the amount of fertiliser added.

The first series of field determinations was made at Rothamsted on a spring barley fertiliser comparison. The results showed considerable fluctuations in nitrate contents, but neither with 0·2 nor 0·4 cwt. of nitrogen per acre was there any evidence that Calcium Cyanamide caused an appreciable retardation of nitrification (Fig. 7). This result provides a marked contrast to that in the pot cultures represented in Fig. 5, which were conducted at the same time.

The second field series was designed to secure greater precision, and the sampling technique was greatly improved. The object was to ascertain whether Calcium Cyanamide caused appreciable delay in nitrification when used during the winter. It has been suggested that the low bacterial activity of the soil in winter might allow urea or ammonia to remain for longer periods, and that the toxicity of cyanamide or its products to nitrifying organisms might reduce the loss of nitrate by leaching. The experiment was laid out as a series of replicated small plots on uncropped land at Rothamsted at the same time as the pot experiment with undisturbed and aerated pots. The results in Table IV show that ammonification of the Calcium Cyanamide was rapid; two-thirds of the added nitrogen was recovered as ammonia after 4 days and urea formed the remaining third. After 3 weeks all the urea had disappeared, thus disposing of the suggestion that cyanamide nitrogen can accumulate as urea throughout the winter. After 3 weeks the ammonia content was about the same as that from ammonium sulphate and the subsequent disappearance of the ammonia was the same from both materials. This again is opposed to the results of the pot experiments, although it may be noted that the "aerated" pots differed less from the field than did the undisturbed soils. The nitrate accumulation was masked by losses through heavy leaching, and although at 3 weeks the nitrate from Calcium Cyanamide was below that from ammonium sulphate, the fact that both the ammonia and the nitrate contents from ammonium sulphate and Calcium Cyanamide were about the same as on unmanured land after 11 weeks would suggest that nitrification went on relatively quickly in both cases. The results for the second depth

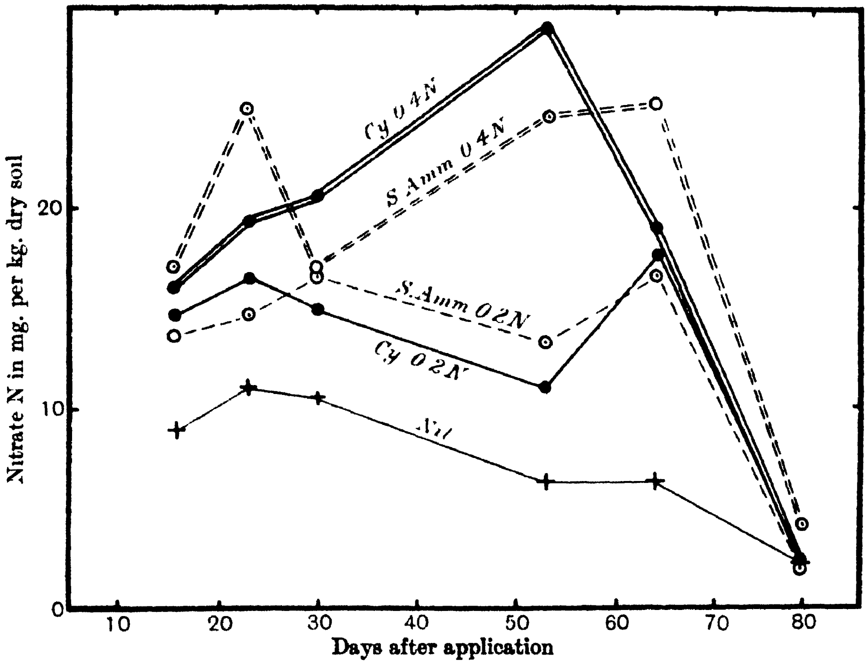


Fig. 7. Nitrate contents of soils in Rothamsted field experiment on barley 1928, for plots without nitrogen or treated with Calcium Cyanamide or ammonium sulphate at rates of 0·2 and 0·4 cwt. N per acre. (R. G. H. Wilshaw's data.)

sampled (10–20 cm.) were similar to those of the surface soil and confirm the downward displacement of the nitrate formed.

Table IV. *Ammonia and nitrate contents of uncropped field soils during winter (treated plots received* 0·4 *cwt. N per acre). H. L. Richardson's data.*

	Soil sampled 0–10 cm.				Soil sampled 10–20 cm.			Soil sampled 20–40 cm.	
	Nov. 9	Nov. 26	Jan. 21	Mar. 25	Nov. 26	Jan. 21	Mar. 25	Jan. 21	Mar. 25
Days after application of fertiliser	4	21	77	140	21	77	140	77	140
Ammonia N in mg./kg. dry soil:									
No nitrogen	5·6	6·2	14·0	4·2	—	—	—	—	—
Ammonium sulphate	50·6	32·0	6·1	6·1	—	—	—	—	—
Calcium cyanamide	33·7	32·8	8·8	3·7	—	—	—	—	—
Urea N in mg./kg. dry soil:									
Calcium Cyanamide	12·5	0·0	—	—	0·0	—	—	—	—
Nitrate N in mg./kg. dry soil:									
No nitrogen	2·3	1·5	2·2	4·2	2·1	2·6	3·7	2·0	2·8
Ammonium sulphate	4·1	8·3	1·7	5·8	9·0	2·4	4·5	4·2	3·2
Calcium Cyanamide	3·1	3·4	3·6	4·9	4·0	5·1	3·2	4·8	4·3

The slight lag in the nitrification of Calcium Cyanamide revealed by these data is clearly insufficient to conserve any appreciable fraction of the added nitrogen in such a way that it becomes available in the surface soil in the following spring. In English practice the application of Calcium Cyanamide to bare land in autumn or winter is rarely likely to occur, though it is sometimes adopted on the Continent in preparation for sugar beet. A further winter experiment was therefore conducted on grassland as a part of an investigation into the behaviour and utilisation of nitrogenous dressings on pastures.

The field (Stackyard) was sown down to grass in April, 1928, with the following mixture sown under winter oats: 16 lb. perennial rye grass, 10 lb. cocksfoot, 4 lb. timothy, ½ lb. rough-stalked meadow grass, 4 lb. late-flowering red clover and 1 lb. wild white clover per acre. It was grazed in 1929. The experiment consisted of six randomised blocks and included the four treatments: winter Calcium Cyanamide (applied Dec. 6, 1929), winter ammonium sulphate (applied Dec. 6, 1929), spring ammonium sulphate (applied Feb. 18, 1930) and no added nitrogen. All fertilisers supplied 0·4 cwt. N per acre. Soil samples to 20 cm. were taken at fortnightly intervals at fifteen points on each plot and the mixed cores for each plot analysed independently. The extracts for

Carsten Olsen's acid salt method for ammonia and nitrate were made within a few hours of taking the samples. The mean results of the soil analyses are plotted in Fig. 8 to provide a further comparison with the earlier experiments; the yields and composition of the herbage will be discussed elsewhere.

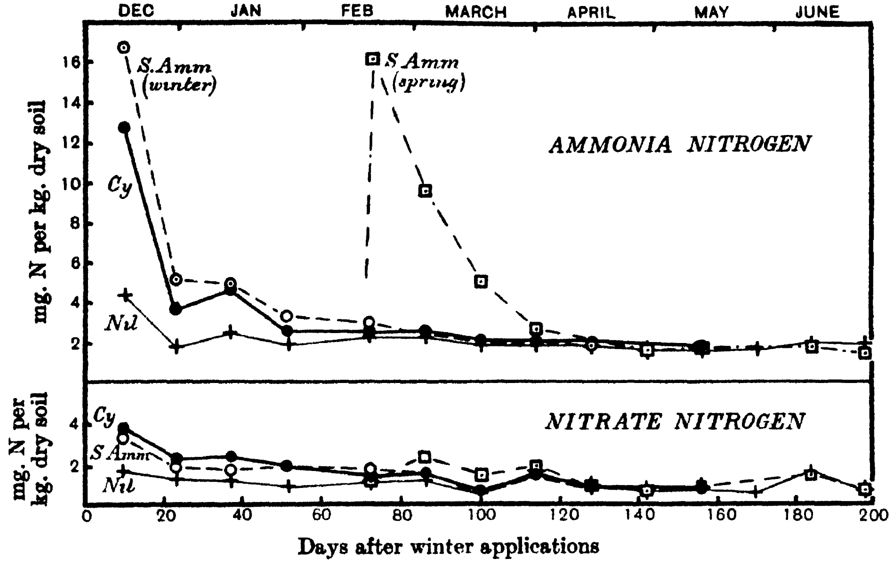


Fig. 8. Ammonia and nitrate contents of pasture soil treated with Calcium Cyanamide in winter and ammonium sulphate in winter or early spring at rates of 0·4 cwt. N per acre. (H. L. Richardson's data.)

Although no attempt was made to work the fertilisers into the soil beyond a light raking, they were washed in by rain which followed shortly after application. Approximate analyses made 3 days later (but not plotted in the figure) showed that the mean ammonia content of plots with Calcium Cyanamide was not much below that of plots with ammonium sulphate. The figure shows that the same difference was found for several weeks, though for all treatments the ammonia contents fell off rapidly. There was thus no evidence of delay in the disappearance of ammonia from Calcium Cyanamide such as would be expected from a nitrification lag similar to that found in the pot experiments. Little nitrate accumulated from any treatment, and throughout the year the nitrate content never exceeded 4 mg. N per kg. soil. It is, of course, impossible to say whether the ammonia was absorbed directly by the plants and micro-organisms or whether it was converted into nitrate which was either absorbed or leached away. It will be noted that during the first few weeks the cyanamide plots had a little more nitrate than the ammonium sulphate plots suggesting a slight checking of growth and nitrate uptake. The recoveries of nitrogen in the first two cuts of the season were 35 per cent. for the spring ammonium sulphate, 14 per cent. for the winter ammonium sulphate and 9 per cent. for the winter Calcium Cyanamide dressings. It would appear therefore that most of the winter nitrogen was either lost by leaching or locked up so firmly by micro-organisms that it did not become available to the plants by the period of active spring growth.

This grassland experiment fully confirms the conclusions from the earlier one on bare land that the retardation of nitrification by Calcium Cyanamide is not sufficient to enable winter applications to supply available nitrogen in spring under such conditions as those at Rothamsted.

THE INFLUENCE OF CALCIUM CYANAMIDE ON SOIL REACTION.

In countries consuming large amounts of Calcium Cyanamide great importance is attached to its basicity and its value in supplying lime, especially for slightly acid sands and light loams. The commercial product contains calcium equivalent to about 60 per cent. CaO, of which about 20 per cent. is present as calcium oxide.

The effect of a dressing of Calcium Cyanamide or of any other nitrogenous fertiliser on the replaceable calcium or reaction of the soil cannot be evaluated in general terms. The amounts of nitrogen and lime absorbed by the crop and the extra amounts of calcium bicarbonate leached away as the result of increased carbon dioxide production from root growth and the decomposition of stubble necessarily vary widely with the crop, the soil, and the environmental conditions; and, in any case, the comparison of a fertiliser against no fertiliser has little practical interest. The relative effects of fertilisers supplying the same amount of nitrogen are of much greater importance and may be calculated, provided it is assumed that they are used under conditions in which they have equal effects on the size and composition of the crop (cf. (10)). Such an assumption may safely be made for normal practice, but not for long-continued fertiliser trials in which marked secondary effects such as crop failure from excessive acidity are introduced. In evaluating the relative effects of nitrogenous fertilisers, calcium or sodium nitrate forms a convenient standard; when nitrogen is added in other forms the ammonia is held by the soil until it is nitrified and the nitrogen not absorbed by the crop or micro-organisms is leached away wholly as nitrate. The extra loss of lime caused by any nitrogenous fertiliser other than nitrates will therefore be equivalent to the excess of its anions (excluding oxide, hydroxide or carbonate) over its kations, when all nitrogen is regarded as nitrate and all carbon and phosphate are ignored. On this basis pure calcium cyanamide has the same effect as equivalent calcium nitrate, and the free lime in commercial Calcium Cyanamide represents the net gain. Ammonium sulphate or chloride, on the other hand, causes an extra loss relative to the nitrate of 2 moles of CaO per mole $(NH_4)_2SO_4$ or per 2 moles NH_4Cl.

That is to say, 100 parts of commercial ammonium sulphate increase the loss of lime by 82 parts of CaO or 147 parts of $CaCO_3$ above that with calcium nitrate or sodium nitrate. The gain from using 100 parts of Calcium Cyanamide instead of 100 parts of ammonium sulphate is therefore (82 + 20 =) 102 parts of CaO or 183 parts of calcium carbonate or, in other words, a dressing of Calcium Cyanamide is equivalent to one of an equal weight of ammonium sulphate together with an equal weight of quicklime. A gain of 1 cwt. of lime per acre is difficult to measure in a single year's experiment and is insufficient to offset the normal loss of lime by leaching in any but the most acid soils. It becomes, however, of considerable importance where heavier dressings are given or when Calcium Cyanamide is used frequently or in conjunction with other basic fertilisers such as basic slag.

The actual advantage in practice may even exceed that estimated on the above considerations when the basic or physiologically alkaline fertilisers are used systematically. Not only is the lime supplied in a very finely divided form in intimate contact with nitrogen or phosphorus at the time of greatest need for lime, but in addition, the lime content of the soil is never increased sufficiently to allow rapid losses. Small frequent dressings of lime in any form are utilised much more efficiently than the heavier occasional dressings which are (or should be) used to counteract the acidification caused by ammonium salts. In a recent examination of the continuous fertiliser and lime plots at Woburn, E. M. Crowther and J. K. Basu (10) showed that sodium nitrate conserved the replaceable calcium of the soil, and that the amount of lime required in occasional limings to raise the replaceable calcium content of soil treated with ammonium sulphate to that with sodium nitrate was much greater than the lime equivalent to the total amount of ammonium sulphate. Calcium cyanamide is necessarily still more effective than sodium nitrate.

The *p*H values of the soils in the pot experiments discussed here and in a subsequent paper (11) were determined after harvesting the barley with results given in Table V. To ensure good growth of barley the acid

Millstone Grit soil received calcium carbonate at the commencement of the experiments. The effects on reaction were slight. Ammonium sulphate consistently reduced the *p*H value, but Calcium Cyanamide did not increase it. On the average the soils with Calcium Cyanamide had *p*H values about 0·2 higher than those with ammonium sulphate. Since most of the soils contained some calcium carbonate, only small differences were to be expected, and those observed probably depended on differences in soluble-salt contents rather than on changes in the degree of saturation of the soil colloids with replaceable bases. Experiments on the acidification or neutralisation of soils under laboratory or pot-culture conditions in which the all-important factor of leaching is omitted have little bearing on the ultimate effect of fertilisers on the reaction and base content of field soils in humid regions. Unfortunately we have no field experiments of sufficient duration to provide a good practical test of the relative effects of Calcium Cyanamide and ammonium salts. We are of opinion, however, that the practical preference for basic or the so-called physiologically alkaline fertilisers is well founded for light soils which are not sufficiently acid to require large dressings of lime. The repeated use of Calcium Cyanamide and basic slag should be more effective in conserving lime than occasional dressings of lime equivalent to the total calcium content of these fertilisers. Such a recommendation clearly only relates to the efficient use of the fertilisers. There are many cases in which a farmer might be better advised to use extra lime and have a free choice of fertilisers.

Table V. *Reaction of soils after pot experiments.*

		Millstone Grit (with $CaCO_3$)			Rothamsted					
Treatment		1926	1928	1929	1926	1928	1929 Hoosfield soil	1929 Broadbalk soil	Woburn 1928	Leagrave 1928
No nitrogen (O.)		6·42	6·84	6·73	8·68	7·63	7·67	7·00	7·53	8·60
Ammonium sulphate (S.A.)		6·21	6·36	6·50	8·55	7·26	7·46	6·45	7·44	8·49
Calcium Cyanamide (Cy.)		6·68	6·50	6·87	8·72	7·53	7·74	6·79	7·50	8·49
		Effect of sulphate of ammonia								
	Mean									
S.A.–O.	−0·26	−0·21	−0·48	−0·23	−0·13	−0·37	−0·21	−0·55	−0·09	−0·11
		Effect of Calcium Cyanamide								
Cy.–O.	−0·03	+0·26	−0·34	+0·14	+0·04	−0·10	+0·07	−0·21	−0·03	−0·11
Cy.–S.A.	+0·23	+0·47	+0·14	+0·37	+0·17	+0·27	+0·28	+0·34	+0·06	0

(The values given are means of determinations on individual pots usually replicated six times per treatment.)

SUMMARY.

1. The work described in the present series of papers was undertaken with the object of ascertaining the best conditions for the use of calcium cyanamide and of comparing it against older nitrogenous fertilisers. Earlier work has in part been repeated so as to ascertain whether the recent improvements in manufacture have overcome the difficulties encountered with the pre-War product.

2. The decomposition of cyanamide to urea can be brought about by a number of minerals likely to occur in the coarser fractions of the soil. The decomposition of cyanamide by soil proceeds according to a logarithmic law such that the rate of disappearance is proportional to the concentration of cyanamide in the soil water.

3. Although soils differ markedly in the rate at which they decompose calcium cyanamide, very few are deficient in the requisite catalyst, and conversion to urea and ammonia is completed within a few days, provided that the calcium cyanamide is intimately incorporated with the soil.

4. The toxicity to germinating seeds is caused by cyanamide itself. It falls off rapidly as the interval between applying the calcium cyanamide and sowing the seeds is increased and is roughly proportional to the amount of cyanamide present during a short interval after sowing.

5. Pot experiments with a range of soils showed that calcium cyanamide decomposed most rapidly in soils of high microbiological activity. The early stages of the decomposition proceeded so rapidly that within a few days the ammonia contents were practically the same whether nitrogen was added as ammonium sulphate or Calcium Cyanamide. The final stage of nitrate formation proceeded more slowly in soils treated with cyanamide.

6. The extent of the retardation of nitrate formation from calcium cyanamide depended on the type of soil and the environmental conditions. In pot experiments it was reduced by improved aeration and in field experiments it was slight in winter and negligible in spring.

7. Under comparable conditions a dressing of calcium cyanamide should have the same effect on the lime supply of the soil as an equal weight of ammonium sulphate together with an equal weight of quicklime. In the regular use of calcium cyanamide there is the additional advantage that its lime is utilised efficiently.

APPENDIX.

Methods of analysis for soils.

In the course of the present series of investigations many alternative analytical methods were tested and developed. Those used for the analysis of soils treated with cyanamide and similar materials are summarised here and those for Calcium Cyanamide, either alone or in fertiliser mixtures, are given in the Appendix to the third paper.

Cyanamide, dicyanodiamide, urea and nitrate are removed completely from soil by extracting with water. The standard method was to extract 250 gm. of fresh soil, passing a 3 mm. sieve, with small quantities of distilled water on a Buchner funnel until some 400 c.c. of extract were obtained. Aliquots from 500 c.c. were used for the following analyses.

Cyanamide nitrogen was determined by a combination of the Caro (12) and Brioux (13) methods. 100 or 200 c.c. of extract were treated with 1 c.c. of concentrated ammonia solution and a slight excess of ammoniacal 10 per cent. $AgNO_3$. After standing, the silver cyanamide was filtered off, washed with 200–300 c.c. of water and nitrogen determined by the Kjeldahl method. (In all Kjeldahl determinations on cyanamide dilute sulphuric acid should be used to ensure full hydrolysis.)

For more rapid work in connection with the germination experiments Pinck's method (14) of dissolving the silver cyanamide in dilute HNO_3 and titrating with KCNS was used. The colloids in the soil extracts prevented the flocculation of the AgCNS and were therefore removed by alum and ammonia, the supernatant liquid being taken for the cyanamide determination.

Dicyanodiamide nitrogen. The newer methods (15) available for the fertiliser mixtures are not suitable for the concentration found in treated soils and the analysis was difficult and unsatisfactory. A modification of Caro's original method (12) was employed. Silver nitrate and sodium hydroxide precipitate the silver derivatives of dicyanodiamide and cyanamide together with silver oxide; total nitrogen is determined in the precipitate and the dicyanodiamide nitrogen is obtained by subtracting the cyanamide nitrogen determined separately. The method is capable of determining small quantities but urea, guanylurea, guanidine and other products are included in the precipitate. A modification of Hene and Van Haaren's method (16) of redissolving and reprecipitating was used thus eliminating urea but not guanylurea. In soil extracts a blank (small for Rothamsted soil, but as much as 0·3 c.c. 0·02 *N* acid for 200 c.c. extract from a fen soil) was needed to correct for the soluble soil nitrogen carried down in the precipitate.

Urea nitrogen was determined by hydrolysing it to ammonium carbonate by Soya Bean, or better, Jack Bean flour, as a source of urease. Soya bean meal was added directly to the soil and ammonia determined by Matthew's aeration method (17). 25 gm. of soil with 1 gm. of Soya Bean flour or 0·5 gm. Jack Bean flour were moistened and left to stand 2–3 hours at ordinary temperatures in the tubes before aeration. Separate ammonia determinations on the soil and on the bean flour are required.

Fox and Geldard's method (18) of adding a fresh urease preparation to a neutral solution followed by direct titration of the ammonia was also used. Extracts prepared as described above gave no difficulty when used directly, but in extracts prepared for Harper's modification (19) of the phenoldisulphonic acid method for nitrates, it was necessary to remove calcium and magnesium by the addition of sodium carbonate and filtration, followed by the addition of strong acid and vigorous aeration to remove carbon dioxide.

Fresh quantities of urease solution were prepared each day by shaking 2·5 gm. Jack Bean flour with 50 c.c. of water for 15 min., neutralising to methyl red, and filtering through a fluted hardened filter paper. 50 c.c. of soil extract were acidified, aerated to remove CO_2, brought to the methyl red end point, and allowed to stand for 1 hour in a stoppered vessel after adding 1 c.c. of neutral urease solution. Excess 0·02 *N* acid was added with a few drops of capryl alcohol to prevent foaming and, after aerating vigorously for 5–10 min. to remove CO_2, the mixture was titrated with 0·02 *N* alkali.

Ammonia nitrogen. Matthews' aeration method (17) was used for all of the determinations except on certain field samples. When appreciable amounts of cyanamide or urea are present in the early stages of an experiment, there is considerable risk of their hydrolysis to ammonia in distillation from alkaline solutions. In later stages, when the cyanamide and urea are known to have disappeared, the aeration method may be replaced by a salt extraction and distillation method with the advantage that nitrates may be determined on the same extract. For this purpose Carsten Olsen's method (20) was adopted with the minor modifications of using NaCl instead of KCl, and bromo-cresol green as indicator. 100 gm. of fresh soil were shaken for 1 hour with 200 c.c. of *N* NaCl containing enough HCl to give a *p*H of about 1·0 in the final suspension. The solution was filtered at once through a large fluted filter (Whatman No. 1, 25 cm. diameter), the first 25 c.c. being discarded to avoid adsorption or contamination by the filter. Distillation was performed as soon as possible, but it could be postponed for a day or so if toluene were added to the original mixture to minimise micro-organic activity. 100 c.c. were diluted with 200 c.c. water and distilled with 3–4 gm. of fresh magnesia into 0·02 *N* acid. The necessity for boiling off CO_2 was avoided by titrating to the end-point given by bromo-cresol green in a buffer solution at *p*H 4·7.

Nitrate nitrogen. In the earlier analyses the water extracts were boiled for 6 hours with alkaline permanganate (10 c.c. of 8 per cent. caustic soda and 10 c.c. of 3 per cent. $KMnO_4$) and then distilled with Devarda's alloy (3 gm.) and 20 c.c. of 40 per cent. NaOH with 5 c.c. of alcohol (21). Cyanamide was removed by a preliminary precipitation as the silver salt as recommended by Jacob (22).

Later, for extracts by Carsten Olsen's method, the residue from the ammonia distillation was redistilled with 2·5 gm. of powdered Devarda's Alloy and 200 c.c. of water. A highly efficient spray trap was necessary, especially for distillations from sodium hydroxide, and a column of small flint pebbles inserted within the neck of the flask was found convenient.

Before adopting the Carsten Olsen's method several series of analyses were made by colorimetric methods. The diphenylamine method (23) was not found satisfactory, and in the phenoldisulphonic acid method the older clearing agents gave trouble either by inefficient clearing or by causing a loss of nitrogen or undesirable tints when the reagent was added. Harper's modification (19) was adopted. The extracts were prepared by shaking 50 gm. of soil for 15 min. with 250 c.c. of distilled water containing 5 c.c. of molar $CuSO_4$ solution. 0·4 gm. $Ca(OH)_2$ and 1 gm. $MgCO_3$ were added and the shaking continued for a further 5 min.; after filtration a suitable aliquot was evaporated to dryness for the colorimetric determination. In the analysis of the extracts from the porous tube experiments in pots it was found sufficient to add 2 c.c. of lime water to a fixed volume of the extract and evaporate to dryness. The colorimetric method has the advantage of speed, but even with the above technique some soils gave uncertain results and the extraction distillation method is much to be preferred.

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STUDIES ON CALCIUM CYANAMIDE[1].

II. MICROBIOLOGICAL ASPECTS OF NITRIFICATION IN SOILS UNDER VARIED ENVIRONMENTAL CONDITIONS.

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(With Four Text-figures.)

THE work described in this paper was undertaken with the double object (*a*) of ascertaining to what extent the characteristic differences in behaviour of calcium cyanamide and ammonium sulphate in moist soil depended on their effects on the microflora of the soil and (*b*) of devising a laboratory technique for following the biochemical and microbiological changes in soils under conditions approximating more closely to those of the field than are obtained in the conventional tumbler technique or in pot cultures. A preliminary series of experiments in uncropped pots showed that periodic sampling of the soil greatly changed the course of the ammonification and nitrification processes presumably by improving the conditions of aeration.

It was shown in the preceding paper (1) that whereas Calcium Cyanamide (the commercial form of calcium cyanamide) caused a considerable lag in the accumulation of nitrate in soils in pot culture, no such lag was found in field experiments conducted at the same time in a similar soil. A series of comparative tests on the changes in bacterial numbers, ammonia, and nitrate contents was therefore carried out in soil receiving Calcium Cyanamide, ammonium sulphate or no nitrogen under a range of conditions of aeration of the experimental soils. An essential feature of the experiments was the frequency of the bacterial counts which were always made at daily or two-daily intervals, since previous work in these laboratories had shown that, both in the field and under laboratory conditions comparable with those in the field, these bacterial numbers fluctuate violently from day to day or even more frequently. Conclusions based on counts at weekly or wider intervals may give quite misleading results. Kühn and Drechsel (2) noted an increase in bacterial numbers after the addition of Calcium Cyanamide, but this was from isolated counts after a month and then at fortnightly intervals.

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[1] This paper was abridged from a portion of the author's "Thesis approved for the Degree of Doctor of Philosophy in the University of London," after his return to India. E.M.C.

All of the experiments in soil in the present work were on a Rothamsted heavy loam soil which had been cropped but unmanured for a long period of years. It was mixed with 10 per cent. of sand and received a basal dressing of 0·2 gm. of potassium phosphate per kg. and nitrogenous fertiliser at the rate of 40 mg. N per kg. of dry soil.

EXPERIMENTS IN POTS.

The usual Rothamsted glazed earthenware pots (36 cm. deep and 17 cm. diameter) with 10 kg. of soil were set up with soil treated with either Calcium Cyanamide, ammonium sulphate or no nitrogen in the winter of 1929–30. The pots—ten for each treatment—were kept in an unheated greenhouse and were uncropped. Daily soil samples for bacterial counts were taken from a pot with each treatment by boring throughout the entire depth of soil with a sterile auger. Bacterial counts were made by plating on Thornton's agar medium, ammonia was determined by Matthews' aeration method and nitrate by Harper's phenoldisulphonic acid method.

Seven samplings were made on successive days from one pot for each treatment, and on every fifth day a new hitherto undisturbed set of pots was sampled in addition. At the times of overlapping, six pots, two per treatment, were sampled and analysed side by side for ammonia and nitrate, so that they were strictly comparable apart from the disturbance and increased aeration of one set for the 4 or 5 preceding days. The nitrogen data were given in the earlier paper (1), and it was shown that in the disturbed pots ammonia was produced from Calcium Cyanamide more rapidly and more completely than in the undisturbed pots and, further, that ammonia, whether added as sulphate or produced in the soil from Calcium Cyanamide, disappeared more rapidly and nitrate was produced rather more rapidly in the disturbed pots.

Table I gives the mean bacterial numbers for the seven daily samples from the individual pots. The general level of numbers was low at the commencement and fell to less than 5 million per gm., presumably on account of the low temperature (mean 8·2°). Calcium Cyanamide gave higher numbers than ammonium sulphate or no nitrogen in six out of eight pots with an average increase of 10 per cent. It was noted that during the seven successive daily samplings there was a general tendency for the numbers to rise to a maximum at the fourth day, especially in the early stages of the experiment. The means for the successive daily counts in individual pots for the whole experiment were 5·5, 5·9, 6·0, 8·0, 7·2, 6·4, 5·1 millions per gm. respectively. These figures apparently reflect the increased bacterial activity resulting from the improved aeration through boring auger holes for sampling. The daily counts fluctuated widely, but neither the five-day means nor the actual counts at the time of sampling revealed any consistent relationship with the changes in ammonia or nitrate nitrogen.

Table I. *Mean bacterial numbers (million per gm.) for seven samplings on successive days on individual uncropped pots with a fresh pot every 5 days.*

	Days from commencement								
	0–7	6–12	11–17	16–22	21–27	26–32	31–37	36–41	Mean
Calcium Cyanamide	9·5	8·8	8·8	4·7	4·3	4·9	5·8	6·6	6·7
Ammonium sulphate	12·4	8·0	5·5	3·6	4·7	4·1	4·6	5·8	6·1
No nitrogen	10·5	7·5	6·5	5·2	4·9	4·4	3·9	5·7	6·1
Mean	10·8	8·1	6·9	4·5	4·6	4·5	4·8	6·0	6·3
Calcium Cyanamide relative to ammonium sulphate = 100	77	111	160	131	92	119	127	116	110

EXPERIMENTS IN FLASKS.

To test the effect of renewal of the atmosphere above the soil, 600 gm. lots of soil were uniformly moistened with 125 c.c. of water applied as a spray and put up in 2000 c.c. Erlenmeyer flasks. For each treatment two flasks were kept corked except at the time of sampling, and in two the air was continuously changed by aspiration. Aerated and non-aerated flasks were sampled for analysis on alternate days. The sampling involved thorough mixing of the soils and the comparison was therefore between intermittent aeration by mixing against the same mixing with constant renewal of the air over the soil mass. The bacterial numbers are given in Table II as the means of five or more counts at two-day intervals, and the nitrate contents as means of two determinations at from five- to seven-day intervals.

Table II.

	Bacterial numbers (millions per gm.) (Means of 5 to 8 counts)				Means of 25 counts	Nitrate content (mg. N per kg. dry soil) (means of two determinations at times shown)			
Days	1–10	11–20	21–33	34–48	1–48	4, 10	15, 20	26, 33	41, 48
Calcium Cyanamide with air current	22	20	15	15	18·3	11·3	15·3	25·5	25·4
" " without air current	21	21	12	12	16·3	10·7	12·2	23·5	25·7
Ammonium sulphate with air current	15	18	12	12	14·1	15·6	25·8	29·6	29·7
" " without air current	14	16	10	10	12·7	15·0	18·8	27·6	30·8
No nitrogen with air current	18	17	11	10	14·1	8·7	8·4	8·9	10·0
" without air current	15	12	12	11	12·4	8·9	7·1	7·1	10·2

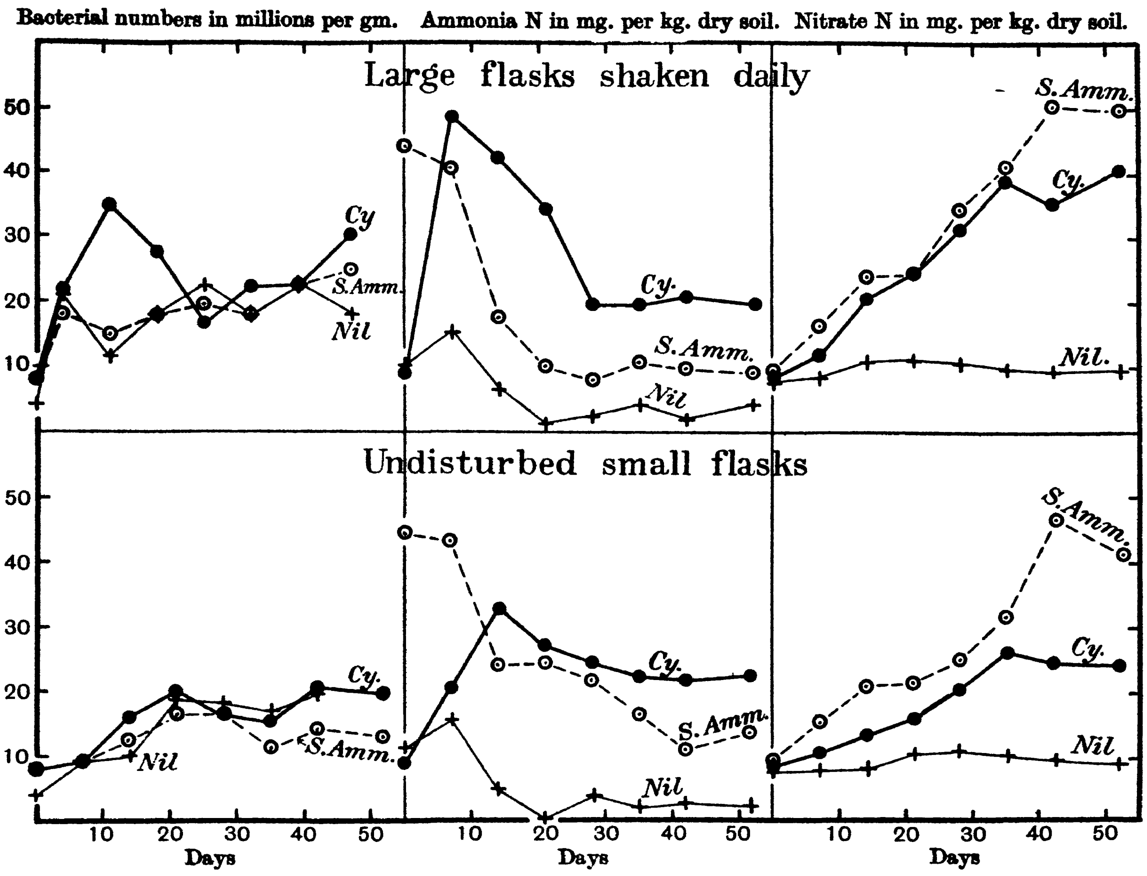

Fig. 1. Changes with time in bacterial numbers (7-day means), ammonia and nitrate in Rothamsted soil treated with Calcium Cyanamide or ammonium sulphate or untreated. Upper figures for large flas shaken daily. Lower figures for small undisturbed flasks.

The air current slightly increased the bacterial numbers, and Calcium Cyanamide again gave the highest bacterial numbers at each period. Nitrate formation was relatively slow and incomplete, and Calcium Cyanamide caused an appreciable lag especially during the second and third weeks.

A further series of flask experiments was made with soil moistened to 50 per cent. of its water-holding capacity and kept under more extreme conditions of aeration. In a fully aerated set 500 gm. of soil were placed in 1500 c.c. wide-mouthed Erlenmeyer flasks plugged with cotton-wool and shaken vigorously for 1 min. every day to provide free gas exchange between the soil crumbs and the air above the soil. Samples were taken for bacterial counts three times per week. Bad aeration conditions were obtained by using sufficient soil to fill small flasks (250 gm. in 250 cc. flask) and leaving each flask undisturbed until it was opened for sampling. Each small flask was again sampled 2, 4 and 7 days later, thus giving a series of intermediate conditions as in the pot experiments. In this series the determinations included in addition to the standard ones urea by the addition of Jack Bean flour as a source of urease, ammonia and urea being determined together by Matthew's aeration method and urea obtained by difference from a direct ammonia determination.

Table III.

	Mean bacterial numbers (millions per gm. soil)						Mean nitrate content (mg. N per kg. dry soil) for 28 to 52 days		
		Small flasks not shaken							
Soil treatment	Large flasks shaken daily and sampled 3 times weekly	First sample 0	Second sample +2 days	Third sample + 4 days	Fourth sample +7 days	Mean of 4 samples	Large flasks shaken daily	Small flasks undisturbed. First sample	Small flasks. Fourth samples
Calcium Cyanamide	24 2	15·2	16·1	22 4	13·9	16·9	36·5	23·7	30 7
Ammonium sulphate	18·3	12·7	16·1	16·3	12·3	14·3	43·9	36·1	37·9
No nitrogen	18·0	13·8	15·7	17 2	16·8	16·3	9·5	9·6	9·8
Mean	20·2	13·9	16·0	18·7	14·8	15·8	—	—	—
Calcium Cyanamide for ammonium sulphate = 100	132	120	100	137	113	118	83	66	81

Curves for the weekly bacterial numbers (means of three counts for the large flasks shaken daily and single counts for the small undisturbed flasks) and for weekly determinations of ammonia and nitrate in both series are given in Fig. 1. Mean bacterial numbers for the whole experiment and for nitrates in the second half of the experiment are also given in Table III. Urea accounted for the following percentages of the added nitrogen: in the large flasks shaken daily, 15 per cent. after 2 days and 2 per cent. after 10 days, and in the small undisturbed flasks, 20 per cent. after 10 days and 10 per cent. after 21 days. Defective aeration thus interfered with the ammonification of the urea produced from Calcium Cyanamide.

The bacterial numbers were consistently higher in the larger shaken flasks than in the small undisturbed flasks or in the earlier experiment. On resampling the small flasks which had remained undisturbed and unaerated until the first sampling there was a steady rise in bacterial numbers on the second and fourth days, as in the first series of pot experiments, though the numbers did not reach the mean values in the fully aerated series of flasks. Again Calcium Cyanamide gave the highest bacterial numbers. In the large aerated flasks both the accumulation and the disappearance of ammonia from Calcium Cyanamide proceeded more rapidly than in the small undisturbed flasks. In the early stages of the experiment there was little lag in nitrate accumulation from Calcium Cyanamide behind that from ammonium sulphate in the large flasks, but there was a marked lag in the unaerated flasks and the final recovery was very low. The flasks in which aeration by sampling followed poor aeration gave intermediate results comparable with those in the corresponding pot experiments.

The greater lag in nitrification from Calcium Cyanamide in the poorly aerated flasks bears directly on the discrepancy already mentioned between the results of earlier studies in the field and in pots. The poorer aeration under pot conditions tends to exaggerate the interference of Calcium Cyanamide with the normal microbiological processes of hydrolysis and oxidation, and this exaggerates the difference between Calcium Cyanamide and other nitrogenous fertilisers. In the usual laboratory experiments on soils the aeration conditions are not as poor as in pot cultures, but since the technique with large flasks and daily shaking reproduces more of the characteristic features of the changes in bacterial numbers and nitrate and ammonia contents in the field it is to be preferred to the old methods. This technique has therefore been adopted in these laboratories in subsequent studies on the biochemical changes in soils.

CARBON DIOXIDE PRODUCTION.

The absence of any relationship between bacterial numbers as determined by plate counts and the changes in the amounts of the simple nitrogen compounds pointed to the necessity of employing other methods of measuring microbiological activity in the treated soils. Since the plating method is highly selective, the rate of carbon dioxide production was used as a general integration of microbiological activity. The total carbon dioxide produced in successive intervals of 24 hours was determined by aspirating air at the rate of 4–5 litres per day over 600 gm. of soil in 2000 c.c. Erlenmeyer flasks and absorbing it in 0·1 per cent.

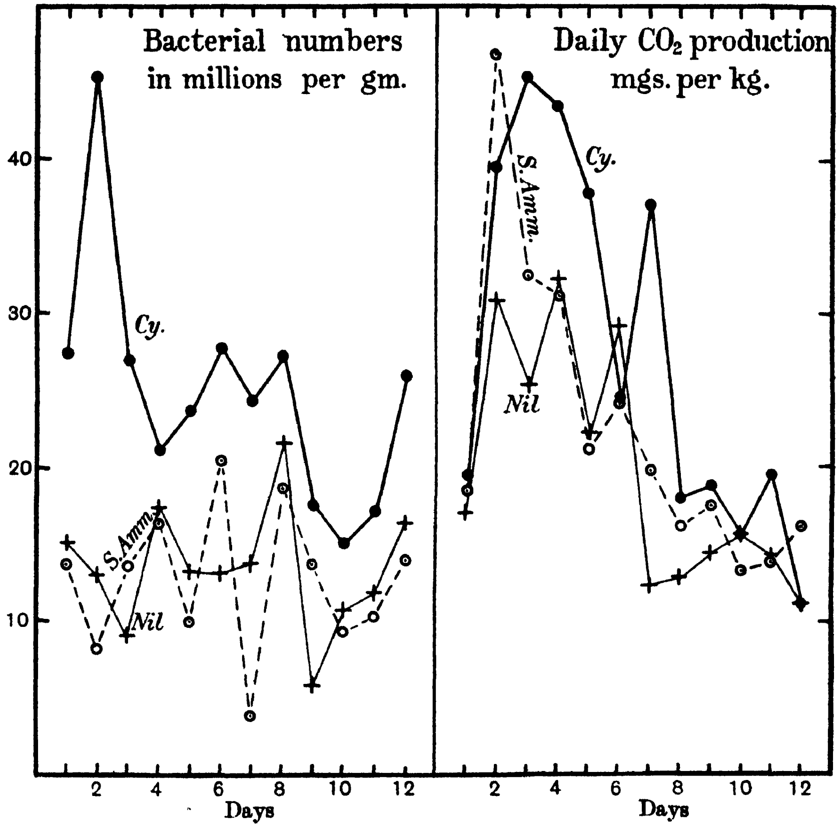

Fig. 2. Daily values of bacterial numbers and carbon dioxide production in soils treated with Calcium Cyanamide or ammonium sulphate or without added nitrogen.

$Ba(OH)_2$ in Pettenkoffer tubes. At the end of each 24 hours' interval samples of soil were taken for analysis. The thorough mixing for sampling ensured good aeration. The daily results of one series of experiments are shown in Fig. 2, and the mean daily carbon dioxide production and the mean bacterial numbers for four-day periods are given in Table IV.

In both experiments Calcium Cyanamide gave much higher bacterial numbers than ammonium sulphate, which itself had no effect on the numbers in the first CO_2 experiment. Both urea and dicyanodiamide gave results similar to Calcium Cyanamide. The curves in Fig. 2 serve to illustrate the rapid fluctuations in bacterial numbers from day to day and the uncertainty of any deduction based on few isolated counts. They also show that there is no significant relationship between bacterial numbers and carbon dioxide production, as was confirmed by the χ^2 test. The increases in bacterial numbers from Calcium Cyanamide, urea or dicyanodiamide are presumably the result of their increasing the ammonifying organisms which are favoured by the plating medium adopted. The Calcium Cyanamide, urea, dicyanodiamide were added at the rate of 40 mg. N per kg. soil, and thus supplied carbon equivalent to 63 mg. CO_2 per kg. soil. The total CO_2 production in excess of that from ammonium sulphate was of the same order (59 and 50 mg. CO_2 per kg. soil for Calcium Cyanamide). Ammonium sulphate had no effect on the bacterial numbers, but slightly increased the CO_2 production.

Table IV. *Bacterial numbers and carbon dioxide production.*

	Bacterial numbers in millions per gm. soil (means of 4 days)			CO_2 production. Daily rate in mg. per kg. soil (means of 4 days)			Total CO_2 production for 12 or 13 days
First exp. (days)	1–4	5–8	9–12	1–4	5–8	9–12	
No nitrogen	14	15	11	26	19	14	238
Ammonium sulphate	13	13	11	32	20	15	271
Calcium Cyanamide	30	26	19	37	29	16	330
Second exp. (days)	1–4	5–8	9–13	1–4	5–8	9–13	
Ammonium sulphate	24	22	12	27	16	9	223
Calcium Cyanamide	33	32	15	27	23	14	273
Urea	42	24	20	31	23	14	285
Dicyanodiamide	34	19	12	36	20	10	276

Apart from any increase in general microbiological activity ammonium sulphate should liberate some CO_2 from a calcareous soil through the productions of acids by nitrification, but in such a well-buffered soil as the one used in these experiments only a fraction of the acid formed would act in this way, and the rest would be taken up by reaction with the exchangeable bases of the soil. In spite of the agreement between the carbon added and the extra carbon dioxide produced it seems unlikely that the increased bacterial numbers depended only on the energy supplied by the hydrolysis of urea; the effects of introducing a soluble and highly reactive nitrogen compound, which, unlike ammonium salts, remains in solution and is not absorbed by the soil colloids, are likely to be much more complex and may result in the oxidation of other carbon compounds present and marked changes in the relative proportions of bacterial and other organisms.

Whatever the mechanism, the results of the whole of the experiments summarised in Table V leave no doubt that Calcium Cyanamide increases the numbers of bacteria capable of growing on the usual counting medium. The following summary also shows that the numbers of bacteria counted increase with improvement in the aeration conditions.

Table V. *Comparison of mean bacterial numbers (millions per gm. soil) for the whole period of experiments with different degrees of aeration.*

	In uncropped pots	Small flasks undisturbed	Large flasks corked and sampled on alternate days	Large flasks with air current and sampled on alternate days	Large flasks shaken daily			Sampled 3 times weekly in sunlight experiment	
					Sampled 3 times weekly	Sampled daily and aerated for CO_2 measurements			
						(1)	(2)	Light	Dark
umber of counts for each mean	56	7	24	24	21	12	13	7	7
alcium Cyanamide	6·7	15·2	16·3	18·3	24 2	24·9	26·3	37·0	46·7
mmonium sulphate	6·1	12·7	12·7	14·1	18·3	12·5	18·7	38·0	41·1
o nitrogen	6·1	13·8	12·4	14 1	18·0	13·4	—	38·0	35·8
lean	6·3	13·9	13·8	15·5	20·2	17·6	—	37·7	40·9
alcium Cyanamide relative to ammonium sulphate = 100	110	120	128	130	132	184	141	98	114

THE EFFECT OF SUNLIGHT ON NITRIFICATION IN SOIL.

Bare fallows, especially in hot, dry periods and in tropical countries are known to increase nitrate accumulation. Opportunity was taken during the bright sunshine of the summer of 1929 to ascertain whether intense light influenced nitrification. The technique with large flasks shaken daily was used, half of them being painted externally with Japan Black. The unpainted flasks being of Pyrex glass admitted light of wave-lengths greater than 3150 Å. Differential heating was prevented by keeping all of the flasks in a trough of cold water.

The results of this experiment differ in several ways from the earlier ones, presumably through the effect of higher temperature. The initial ammonia content of the soil was very high, the bacterial numbers rose much higher, and ammonium sulphate nitrified more rapidly than in the earlier flask experiments. Calcium Cyanamide caused no increase in the bacterial numbers in the flasks exposed to light, and both in light and in dark its rate of nitrification was below that of ammonium sulphate and similar to that of the earlier experiments.

The general effect of the solar radiation was to retard nitrification and the production of ammonia from Calcium Cyanamide and to reduce bacterial numbers (Fig. 3). It seems probable therefore that the increased nitrification observed elsewhere depended on drying or heating rather than on a direct photochemical effect.

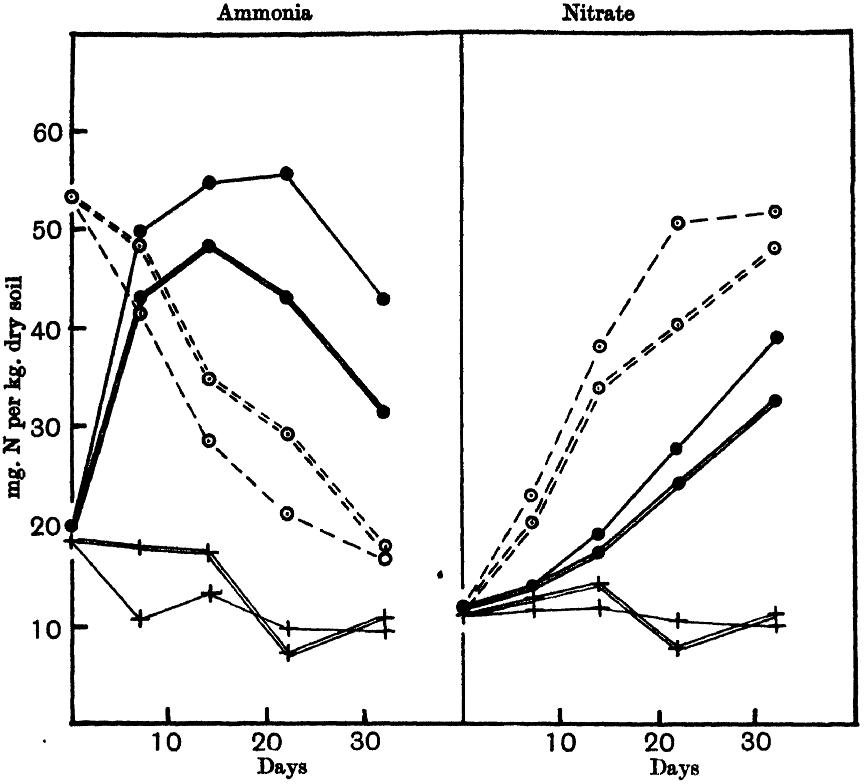

Fig. 3. Effect of sunlight on production of ammonia and nitrate from Calcium Cyanamide and ammonium sulphate. Single lines: soil in darkened flasks; double lines: soil exposed to intense sunlight. Open circles, ammonium sulphate; full circles, Calcium Cyanamide; crosses, no nitrogen.

EXPERIMENTS IN CULTURE SOLUTIONS.

To study the direct effect of the various forms of nitrogen on soil micro-organisms it was necessary to work in culture solutions so as to avoid the rapid decomposition of cyanamide by non-biological processes. The first experiments were made on a mixed soil flora obtained by inoculating 50 c.c. of nutrient solution with 0·2 gm. of fresh soil from a continuous farmyard-manure plot on Barnfield at Rothamsted. The culture solutions were prepared by sterilising separately and mixing equal quantities of the following solutions:

(*a*) 5 gm. ammonium sulphate per litre,

(*b*) 2 gm. potassium phosphate, 1 gm. magnesium sulphate, a trace of calcium chloride, and 0·4 gm. ferrous sulphate per litre, with the addition of 0·5 gm. of magnesium carbonate per flask and additional nitrogen at the rate of 40 mg. per litre in the forms of sterilised solutions of urea or dicyanodiamide (melting point 202° C.) or a suspension of Calcium Cyanamide. One set had no nitrogen beyond the ammonium sulphate (515 mg. N per litre). The flasks were incubated at 23° C. and sampled periodically for determination, after centrifuging, of ammonia by Nessler's method, nitrite by the Griess-Ilsova method and nitrate by the phenoldisulphonic acid method. The nitrite and nitrate contents are given in Fig. 4. Small amounts of nitrite were formed immediately, but no nitrate appeared until the 20th day when it was detected in flasks with ammonium sulphate only. The initial lag in all cases was doubtless caused by the injurious effect of free ammonia. Calcium cyanamide markedly inhibited the formation of nitrite and completely prevented the production of nitrate. Dicyanodiamide retarded both nitrite and nitrate formation. Urea reduced nitrite formation in the early stages only but markedly stimulated nitrate production

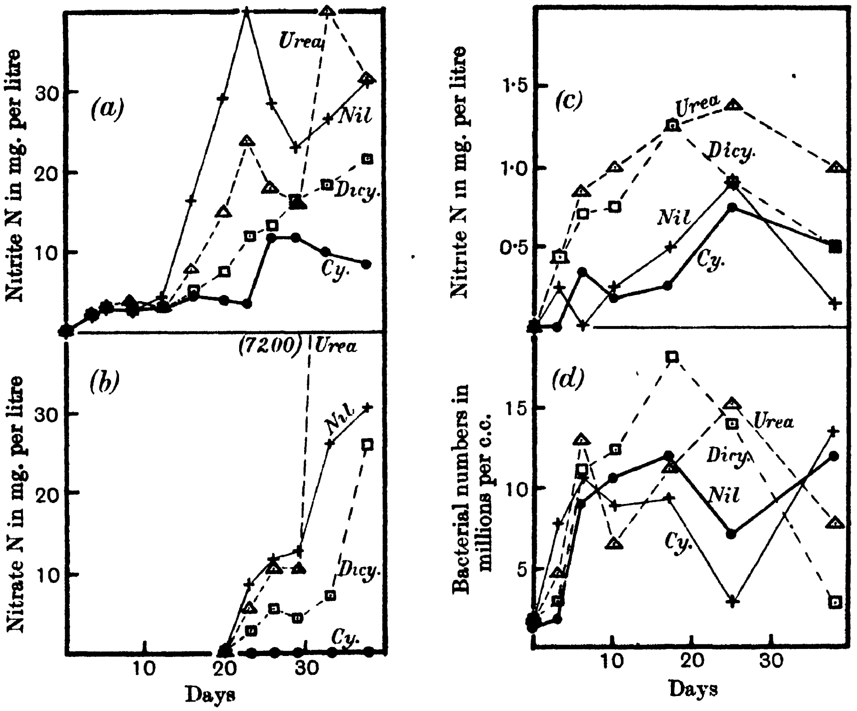

Fig. 4. Influence of Calcium Cyanamide, urea, and dicyanodiamide on nitrification in liquid media containing excess ammonium sulphate. (*a*) nitrite and (*b*) nitrate production by mixed culture from inoculation by soil, (*c*) nitrite and (*d*) bacterial numbers in pure cultures of nitrite producing bacteria (A 2).

towards the end of the experiment. After 5 weeks about 40 per cent. of the total nitrogen from ammonium sulphate was nitrified where urea was present but only about 5 per cent. where ammonium sulphate was used alone. The loss of ammonia from all cultures was much in excess of the nitrite and nitrate formed; some nitrogen was presumably built up into bacterial protoplasm but much may have escaped by volatilisation.

This experiment showed that calcium cyanamide or its immediate products of hydrolysis on solution in water was much more toxic than dicyanodiamide. In the earlier literature relatively little attention was given to the toxicity of cyanamide and it was generally assumed that dicyanodiamide was the active poison. The culture experiments suggest that direct toxicity of cyanamide to nitrifying organisms is sufficient explanation of the retardation of nitrification in soil cultures or in pot experiments even in cases where no dicyanodiamide is formed. The fact that cyanamide proves less injurious than dicyanodiamide to nitrification in soil is of course explained by its rapid decomposition by non-biological process with the production of urea which stimulates both the multiplication of ammonifying bacteria and the production of nitrate.

Attempts were made to examine the direct effects of cyanamide and related substances on nitrifying organisms in soil by direct counts on silica plates and by dilution methods. The numbers obtained were so low as to suggest that organisms capable of growth on the customary silica plate cultures were not responsible for the nitrification observed in the soils. An extension of this work in conjunction with other work in progress on the biological oxidation processes in filters for sugar-beet effluents led to the discovery of many strains of nitrifying organisms which differed from Winogradsky's *Nitrosomonos* and *Nitrobacter* not only morphologically but in their ability to grow and produce nitrite in solutions containing sucrose or on agar plates. These organisms are described elsewhere (3). One of them (A 2) was used in pure culture in mineral salt solution to test the effects of cyanamide, dicyanodiamide and urea on nitrite production from ammonium sulphate. 50 c.c. of nutrient salt solution containing 200 mg. of nitrogen per litre as ammonium sulphate with 40 mg. of nitrogen per litre in the above form were incubated at 23° and periodic samples taken for nitrite determinations and bacterial counts by means of a haemacytometer (Fig. 4). Urea and dicyanodiamide gave much higher bacterial numbers than Calcium Cyanamide or no additional nitrogen. Urea and dicyanodiamide markedly stimulated nitrite production. Calcium Cyanamide again reduced bacterial numbers.

The whole of the experiments agree in showing that although Calcium Cyanamide increases the numbers of bacteria capable of growing on agar plates, it is markedly toxic to nitrifying organisms. Dicyanodiamide is less toxic than cyanamide in equivalent amounts, but in soil cultures it proves more harmful because it disappears from the soil much less rapidly. The further decomposition of the immediate products of cyanamide in the soil depends on the environmental conditions and proceeds more rapidly in well-aerated soils. In the microbiological studies, as in the chemical ones recorded in the preceding paper, the general rule holds that in conditions generally favourable for growth of plants and micro-organisms the decomposition of Calcium Cyanamide proceeds so rapidly that it resembles that from simpler form of nitrogenous fertilisers; under more adverse circumstances, and especially with inadequate aeration, the partial differential soil sterilisation by Calcium Cyanamide reduces the rate of nitrate accumulation.

SUMMARY.

1. Calcium Cyanamide (the commercial form of Calcium Cyanamide) markedly increased the bacterial numbers of soils in uncropped pots and both the bacterial numbers and carbon dioxide production in flasks under laboratory conditions.

2. Improvements in the aeration of soil cultures in the laboratory not only increased the numbers of bacteria and accelerated the production and disappearance of ammonia but reduced the initial nitrification lag and increased the final accumulation of nitrate from Calcium Cyanamide. Urea formed from Calcium Cyanamide disappeared much more quickly in soils aerated by daily shaking than in undisturbed soils.

3. A technique for microbiological and biochemical studies on soils was developed to ensure adequate aeration and it was shown to give results more in accordance with those under field conditions than were obtained by the older methods for soil cultures.

4. Although dicyanodiamide has a greater depressing effect on nitrate formation in soils than Calcium Cyanamide, the latter substance is much more toxic to nitrifying organism in culture solution. In soils the toxic effect of cyanamide is obscured by its rapid decomposition to urea.

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STUDIES ON CALCIUM CYANAMIDE.

III. STORAGE AND MIXING WITH SUPERPHOSPHATE

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Calcium Cyanamide (the commercial form of calcium cyanamide), as prepared by modern methods and marketed in England, consists largely of the pure compound along with some carbon and quicklime resulting from the reactions of its manufacture, and perhaps traces of calcium carbide. It is oiled to reduce dustiness, but as it is not treated with water it contains no measurable amounts of dicyanodiamide. Of its total nitrogen content, about 95 per cent. is recovered on analysis as cyanamide nitrogen and some 1·0–1·5 per cent. as urea nitrogen; the remainder may be in the form of nitrides, chiefly silicon nitride.

The fertiliser is delivered to the farm in double, paper-lined bags, and if applied without delay may be expected to have undergone no change.

It is well known, however, that on storage in an exposed condition Calcium Cyanamide takes up moisture and carbon dioxide from the air; this is accompanied by changes in the material, so that under extreme conditions in the laboratory the cyanamide nitrogen is finally converted almost completely into urea and dicyanodiamide. It has also been found that when Calcium Cyanamide is mixed with superphosphate considerable heat is developed, and again some of the nitrogen is converted to other forms, the extent and nature of the changes varying considerably with the conditions (see especially Jacob and Braham (1)).

Since urea is rather more, and dicyanodiamide much less, rapidly available in the soil than cyanamide itself, and since also dicyanodiamide has been found in laboratory experiments to retard nitrification, these changes may become of practical importance. As earlier investigations referred to products or conditions differing considerably from the modern English ones, the present investigation was undertaken (*a*) to ascertain the extent to which these changes may take place in practice with modern products, (*b*) to throw some light on the factors responsible for such changes as occur.

Storage. On exposure to air, the quicklime takes up water and carbon dioxide to give calcium hydroxide and carbonate. In the moist, strongly alkaline conditions so produced, some of the cyanamide nitrogen is polymerised to dicyanodiamide while some urea is also formed by hydrolysis and traces of other complex products may be produced (2).

The rate at which these changes proceed under ordinary storage conditions on the farm was investigated in a series of eight half-hundredweight, paper lined sacks of Calcium Cyanamide. When received, at the end of March, they were opened, sampled by numerous tube borings to the full depth of the bag, and carefully sewn up again. The percentages of total nitrogen, cyanamide, urea and dicyanodiamide nitrogen were determined in the samples by methods described in the Appendix. The individual bags were weighed and stored in a group in the corner of a brick store-room: they stood on wooden planking on the floor of cemented brick, a few inches above outside ground level. The room was generally dry, but rain sometimes entered through a door not far away. The store had an upper storey which kept the temperature fairly low through the summer. The general conditions were those of good storage on the farm.

Through a total period of storage of more than 2 years, bags (chosen by lot) were weighed and sampled at intervals, to follow the changes in their contents. For these samplings, which were made after 1½, 3, 6, 12 and 27 months, the contents of a weighed bag were emptied on to a sheet, thoroughly mixed, and a sample taken in numerous small portions. The Calcium Cyanamide was then returned to the bag, which was sewn up again, and all the bags replaced at random in the corner. Comparison was made of bags which had not previously been emptied out for sampling, while towards the end some bags were re-sampled for an additional comparison.

The Calcium Cyanamide in the bags tended on standing to settle into an apparently solid mass, which readily broke into powder on handling; there was no formation of hard crust or lumps until after 2 years, when the contents of some of the bags had swelled so much as to burst the sacking or the stitches at the top. A rather soft crust was formed where the Calcium Cyanamide was thus exposed.

The results are summarised in Table I; the weights are mean values for all bags which had not previously been emptied out for sampling at the time of weighing; it was found that after re-bagging the sacks gained slightly more in weight than the undisturbed ones. Where towards the end bags were re-sampled for comparison, the values obtained are shown in brackets.

Table I.

Storage of Calcium Cyanamide (means)

	No. of bags sampled for analysis	Percentages of initial values			Percentages of total nitrogen at time of sampling		
		Weight	Nitrogen %	Total nitrogen content of bag	Cyanamide nitrogen	Urea nitrogen	Dicyanodiamide N
Initial	8	100	100	100	94·9	1·1	Nil
After 1½ months	2	100·6	99·5	100·1	94·8	1·4	Nil
„ 3 „	2	101·6	97·1	98·6	95·6	2·0	Nil
„ 6 „	2	103·6	94·9	98·3	94·6	1·6	0·3
„ 12 „	1	105·5	92·0	97·1	93·7	—	0·6
	(1)	(106)	(95)	(101)	(93·0)		(0·8)
„ 27 „	1	108·1	94·3	102	—	—	—
	(1)	(110)	(89)	(98)			

The results make it clear that the modern bags afford distinctly good protection of their contents, and that the fertiliser may safely be kept for some months, or even a year, in a dry shed on the farm so long as the bags are not opened. The changes should be still less in the commercial 2-cwt. sacks than in the ½-cwt. bags used in these experiments. In a damp shed, however, or in bags that have been opened and not sewn up again, deterioration may be more rapid.

Taking the results in more detail, the weight of the Calcium Cyanamide increased steadily but the rate of increase fell off after the first 6 months; as the material at the surface was changed, the reacting gases had farther to diffuse and the reaction was slowed down.

Nitrogen content (as per cent. of the material or of the initial value) fell off steadily as the material gained in weight, and to an almost corresponding extent. The product of nitrogen content and weight decreased slightly up to 6 months, but with longer storage (when sampling difficulties may have been greater) the effect was not maintained. At no time was the loss of nitrogen sufficient to have any significance in practice.

Cyanamide nitrogen as a percentage of total nitrogen showed no appreciable change up to 6 months, and even after 12 months it had fallen by only 1 or 2 per cent.; *urea* was correspondingly almost unchanged. Dicyanodiamide could not be detected before 6 months and even after 12 months the amount (0·1 per cent. of the fertiliser) was negligible for practical purposes.

Mixing with superphosphate. An obvious indication of some (possibly harmful) interaction during the mixing of Calcium Cyanamide and superphosphate is the evolution of heat that occurs. Attempts have been made to reduce the heating to a minimum, either by spraying the mixture with water or by spreading it out in a thin layer immediately after mixing. The former method has obvious disadvantages, both from its effect on the condition of the mixture and from the likelihood of the extra moisture encouraging deleterious reactions; the latter is now recommended as standard practice where mixing is unavoidable. Although it might be preferable to avoid making this mixture, the convenience of applying fertilisers in a single dressing is so great that it was clearly desirable to know the nature and extent of the nitrogen changes under the standard conditions, and the likelihood of their being deleterious in nature. Subsequently, to throw more light on the reactions occurring, a series of laboratory mixtures of different proportions of superphosphate and Calcium Cyanamide was prepared by A. G. Pollard, who studied the phosphate solubilities of the mixtures and kindly supplied samples for the examination of the nitrogen compounds.

In the first experiment a mixture of 1 cwt. Calcium Cyanamide and 3 cwt. superphosphate was made by hand on the concrete floor of the farm manure shed; it became warm and evolved some steam, but was at once spread in a layer 1–2 in. thick, where it cooled rapidly. It was left overnight, sampled for analysis while being re-mixed, and then put up in 1 cwt. sacks for 1 month. After this period the mixture was emptied out (there had been no formation of crusts or hard caking), re-mixed, and again sampled. The mixture lost 2·7 per cent. of its weight during the first mixing; it gained 0·9 per cent. in weight while standing in bags for a month.

Table II. *Farm mixture of* 3 *parts superphosphate and* 1 *part Calcium Cyanamide.*

	Percentages of mixture.				Percentages of total nitrogen		
	Total N	Cyan. N	Urea N	Dicy. N	Cyan. N	Urea N	Dicy. N
Calculated on mixture (excluding reactions)	4·6	4·4	0·05	0	95	1·1	0
Found after 16 hours	4·5	3·3	0·13	0·74	73	2·9	16·5
Found after 1 month	4·5	3·15	0·25	1·14	70	5·6	25

Owing to the heterogeneous nature of the mixture (which contained many small lumps of unchanged superphosphate), the sampling was difficult and a high degree of accuracy cannot be expected in the values given. The dicyanodiamide values are least subject to this error since they were determined on the largest samples. The results (Table II) show that with the limited degree of heating allowed to take place under the standard conditions, about three-quarters of the cyanamide nitrogen remains unchanged in a mixture of the proportions here used and a little less after a month's storage. The production of urea is slight, even after storage. The chief form into which the nitrogen is changed is dicyanodiamide, one-sixth of the total nitrogen being in this form shortly after mixing, and one-fourth after the month's storage. It is unlikely that any guanylurea would be formed since this mixture had an alkaline reaction. It should be emphasised that the extent and nature of the changes found in superphosphate-Calcium Cyanamide mixtures depend on the proportions and the conditions of mixing, so that for farm mixtures the results would be different from those given if different proportions were used or the pile was allowed to heat up more after mixing.

Some idea of the influence of changed conditions, particularly the use of different proportions, and also information about the general nature of the reactions taking place may be obtained from an examination of mixtures prepared by A. G. Pollard, who supplied the data for temperature rise, moisture content and water soluble phosphoric acid. They were made in 1·5 kg. lots in the laboratory and allowed to heat and cool in vessels protected against rapid heat loss; the dicyanodiamide determinations were made some weeks after the mixtures were prepared, so that they are comparable with the "1 month" values in Table II. The results for this series of mixtures appear in Table III.

Table III. *Changes in a series of superphosphate-Calcium Cyanamide mixtures.*

% of Calcium Cyanamide in mixture	Dicyanodiamide N		Water soluble P_2O_5 % of total P_2O_5	Moisture %	Temp. rise on mixing °C.
	% in mixture	% of total N (calculated)			
1	0·00	0	82·1	13·2	5
3	0·006	1	85·8	12·8	5
5	0·075	7·3	87·3	11·5	15
7	0·157	10·9	76·9	11·8	28
10	0·523	25·6	55·2	11·8	44
20	2·09	51·0	Trace	7·3	74
35	2·51	35·0	Nil	4·5	86
50	1·89	18·4	Nil	2·9	92

Since the chief interest in these mixtures was in the production of dicyanodiamide, and other forms of nitrogen were not determined, a complete discussion of the nitrogen changes is not possible. Dicyanodiamide production, however, proceeded with considerable regularity, rising rapidly to an amount equivalent to 50 per cent. total nitrogen in a mixture containing 20 per cent. of Calcium Cyanamide and then falling steadily with increasing proportions of Calcium Cyanamide. The regularity of this change, together with certain features of the behaviour of the other properties of the mixtures, justify a closer examination of the results.

The primary reaction taking place in these mixtures is the reversion of the superphosphate

$$CaH_4(PO_4)_2 + CaO = 2CaHPO_4 + H_2O.$$

In mixtures with a low proportion of Calcium Cyanamide, containing more than enough monocalcium phosphate to combine with the CaO added, some water is liberated, the excess of monocalcium phosphate remains water soluble and the mixture as a whole retains an acid reaction. In mixtures rich in cyanamide, on the other hand, the excess of CaO uses up some of the moisture from the superphosphate for the formation of $Ca(OH)_2$, while the mixture as a whole has an alkaline reaction and there is no water soluble phosphate. The point at which "reversion" is complete is consequently indicated by a disappearance of water soluble phosphate and a more rapid decrease in moisture content than would be expected from the simple mixture rule.

The rise in temperature on mixing does not show a break at the point of complete reversion, for up to this point increasing amounts of heat are evolved from the reaction with the monocalcium phosphate and subsequently from the slaking of the quicklime.

The water soluble P_2O_5 and moisture content values in Table III show that reversion was complete at some point between the 10 and 20 per cent. Calcium Cyanamide mixtures. In mixtures with 10 per cent. or less of cyanamide, therefore, moist acid conditions existed; in mixtures with 20 per cent. of cyanamide or more, the conditions were increasingly alkaline but with steadily decreasing moisture. In the first paper of this series (2) it was shown, in a review of the chemistry of Calcium Cyanamide, that in dilute solutions under acid conditions cyanamide hydrolyses to urea, under moderately alkaline conditions it rapidly polymerises to dicyanodiamide, and under strongly alkaline conditions the polymerisation is less rapid and some urea accompanies the dicyanodiamide. Some differences are to be expected in the behaviour of moist solid mixtures, for the effects of increasing alkalinity are offset by the reduction in moisture content. Dry Calcium Cyanamide is of course unchanged on heating.

On the whole, however, the production of dicyanodiamide in these mixtures may be interpreted in terms of the progressive changes in other conditions that have been shown to exist. In the "acid" mixtures, up to 10 per cent. Calcium Cyanamide, the proportion of nitrogen converted to dicyanodiamide was small; if any marked change in nitrogen took place it would be the production of urea. In the first alkaline mixture, which was fairly moist and hot, the production of dicyanodiamide was very high (while little urea would be expected). In richer mixtures the proportion of the nitrogen polymerised to dicyanodiamide fell off, in spite of increasing rise in temperature; it is possible that more urea was formed, but probably the production of dicyanodiamide (and still more the hydrolysis to urea) was limited by the decreasing moisture content and most of the nitrogen remained unchanged as cyanamide.

Considering the practical bearing of these results, they may be applied first of all to the bulk mixture (1 cwt. of Calcium Cyanamide and 3 cwt. superphosphate) already described. Since this corresponds with 25 per cent. in the present series, all the phosphate would be reverted and there would be an excess of CaO giving an alkaline mixture. The low production of urea observed was to be expected under these conditions but under the conditions of the laboratory experiment some 45 per cent. of the nitrogen would be converted to dicyanodiamide. The divergence of this from the value (25 per cent.) found in the bulk mixture is a measure of the success of the method of preventing overheating by spreading in a thin layer immediately after mixing.

The relatively low production of dicyanodiamide in 50 per cent. mixtures, even where strong heating was allowed in the laboratory, is noteworthy. It suggests that when the mixing of Calcium Cyanamide and superphosphate is unavoidable on the farm, it is preferable to use mixtures which contain as nearly equal parts of the two constituents as possible. Another deduction that may be drawn is that the superphosphate used should be as dry as possible, since much of the heating effect depends on the slaking of the CaO by moisture from the superphosphate and in addition lack of moisture seems to have limited the production of dicyanodiamide in the high-cyanamide mixtures.

In this discussion it has been assumed that the conversion of cyanamide into dicyanodiamide is undesirable. This is true for crops where rapid availability of the nitrogen is essential, but there are other crops, including probably grassland, for which some rapidly available nitrogen, together with a source of slowly available nitrogen, is preferable. There is a distinct possibility that dicyanodiamide in mixtures may supply nitrogen in this way, either by retarding nitrification or by its own slow availability. For this purpose it might be an advantage to increase the dicyanodiamide production by using mixtures such as 1 Calcium Cyanamide to 4 superphosphate without special provision for cooling.

SUMMARY.

1. On storage under good farm conditions Calcium Cyanamide gained steadily in weight, the increase reaching about 10 per cent. after 27 months. The percentage of nitrogen decreased at a practically equivalent rate, so that there was little change in total nitrogen. The form of the nitrogen remained practically unchanged for the first 6 months; by 12 months there was a slight reduction in cyanamide nitrogen and a slight production of dicyanodiamide—less than 1 per cent. of the total nitrogen.

2. In a farm mixture of Calcium Cyanamide and superphosphate (1 : 3) cooled by spreading in a thin layer after mixing, 16 per cent. of the nitrogen was converted to dicyanodiamide in the fresh mixture and this increased to 25 per cent. after 1 month's storage. More dicyanodiamide was produced in a series of laboratory mixtures of Calcium Cyanamide and superphosphate, in which heating was allowed to take place. Dicyanodiamide production varied regularly with the composition of the mixtures, rising to a maximum of 50 per cent. of the total nitrogen in the mixture containing 20 per cent. of Calcium Cyanamide, and falling to below 20 per cent. of the nitrogen in the 50 per cent. mixture.

APPENDIX.

Analytical methods for fertilisers.

In analysing Calcium Cyanamide, particularly after storage or in mixtures with other fertilisers, especial care was found to be necessary in sampling. In order to secure concordant results the bulk samples of 400 to 800 gm., taken in numerous small portions, were crushed free from large lumps, well mixed, and subdivided by quartering. For the dicyanodiamide determinations, made on from 15 to 100 gm. of material, grinding was not necessary, but for the determinations made on smaller amounts of material—cyanamide, urea, and especially total nitrogen—50–100 gm. obtained by repeated quartering were completely ground to pass a 0·5 mm. sieve and samples taken from this for the actual analyses which were generally made in triplicate. On account of the CaO content of the material it was necessary to reduce exposure to air during sampling and weighing to a minimum.

Total nitrogen. The Kjeldahl method was used, with the important modification of adding sufficient water to the sample for the cyanamide nitrogen to be completely hydrolysed during the first stages of the digestion. If concentrated sulphuric acid was added to the dry material some was decomposed with the evolution of gases smelling of cyanide, and results were low and discordant. For a sample containing sufficient nitrogen to neutralise some 40 c.c. of $N/10$ acid (*i.e.* 0·25–0·30 gm. of

Calcium Cyanamide) 50 c.c. of distilled water, 20 c.c. of concentrated sulphuric acid, 10 gm. of sodium sulphate (crystals), and a pea-crystal of copper sulphate were added. Mercury and its compounds were tested as catalysts, but their use necessitated the precipitation of the mercury as sulphide before distillation, and no increase in accuracy was secured. The digestion flask was heated gently for 1–2 hours until the water had boiled off, and digestion was continued for a further 2–3 hours; subsequently the normal Kjeldahl procedure was followed.

Cyanamide nitrogen. The Caro-Brioux procedure (3) was standardised as follows: an extract of the ground material was made by shaking in a machine for 3 hours; this extract was used also for urea determinations, and dicyanodiamide where this was determined by the difference method. For Calcium Cyanamide 5 gm. was extracted with 500 c.c. of distilled water. The extract was filtered through a fluted filter and determinations made immediately to reduce the risk of change in the alkaline solution. For the cyanamide determinations, aliquots (25 c.c. for Calcium Cyanamide) were transferred to small beakers, 5 c.c. of 1 : 1 ammonia solution added, and a slight excess of $M/10$ $AgNO_3$—actually, several c.c. more than the number of c.c. of $N/10$ acid likely to be required in the final distillation. The mixture was left overnight under a clock glass for precipitation to be completed, filtered with small papers and funnels to reduce the amount of washing necessary, and washed with distilled water until 175 c.c. of filtrate were collected. The funnel and contents were allowed to drain and dry apart from the filtrate (these precautions were taken because of the presence of ammonia in the original solution). The precipitate on the paper was then transferred to a Kjeldahl digestion flask, 50 c.c. of distilled water added, and nitrogen estimated as in the "total nitrogen" procedure.

Urea nitrogen. Fox and Geldard's urease method (4), as described in the Appendix to the first paper of this series, was employed on a portion of the aqueous extracts prepared as in the preceding paragraph. Calcium, and phosphates if present, were first eliminated from the solution by the addition of a little finely powdered $Ba(OH)_2$ and a small excess of anhydrous Na_2CO_3, followed by shaking for half an hour. The mixture was filtered (fluted filter) and aliquots taken, acidified with strong hydrochloric acid (to avoid undue dilution), aerated vigorously to expel CO_2, and made exactly neutral to methyl red. Neutral urease (extract of Jack Bean flour) was added and the ammonia resulting from the urea hydrolysis titrated directly, as already described.

Dicyanodiamide nitrogen. The difference method described in the Appendix on soil analysis was first used, but the disadvantages there noted led to an examination of more direct methods of determination. Silver picrate forms relatively insoluble complex compounds with dicyanodiamide, on which methods have been based (Harger (5), Johnson (6)) but Harger's method was not found satisfactory for very small amounts of dicyanodiamide, and an attempt to use trinitroresorcinol in place of picric acid was no more encouraging.

Finally the nickel guanylurea method worked out by Garby (7), although rather complex in its procedure, was found to give very satisfactory results. Since urea does not interfere in this method and guanylurea salts such as might occur in superphosphate mixtures are almost insoluble in the acetone used as solvent, the procedure determines dicyanodiamide alone, separating it from the compounds that may interfere in the difference method.

This method depends on the hydrolysis in acid solution of dicyanodiamide to guanylurea, and the precipitation of the latter as the almost insoluble nickel derivative. In order to arrest the hydrolysis at the right point, and secure complete precipitation of the nickel compound, careful control of the conditions is necessary. A sufficiently large (15–100 gm.) sample of the Calcium Cyanamide or its mixtures was extracted with acetone (250 or 300 c.c.) by shaking for 2–3 hours, the solution filtered and the acetone distilled off from aliquots of 50 c.c. in small flasks. If appreciable amounts of dicyanodiamide were present a crystalline residue remained. contaminated with oil from the Calcium Cyanamide. This was removed by rinsing with absolute ether, in which dicyanodiamide is insoluble. The residue was dissolved in 20 c.c. of $N/4$ HNO_3, transferred to a small evaporating basin, partly covered with a watch-glass to control the rate of evaporation, and taken to dryness in 1¾ to 2¼ hours on a steam bath. The residue of guanylurea nitrate was dissolved in 40 c.c. of a 10 per cent. solution of mannitol saturated with nickel guanylurea, treated with 3 c.c. of a solution of nickel nitrate (40 gm.) and ammonium nitrate (20 gm.) in 100 c.c. of the mannitol-nickel guanylurea solution and sufficient 20 per cent. NaOH was added drop by drop to produce a greenish yellow colour. (It was found important to reproduce the same colour, *i.e.* degree of alkalinity, as nearly as possible in all determinations.) These operations were conducted and the solutions left in small weighing bottles with ground stoppers to prevent loss of ammonia. The solutions were filtered after standing overnight, using sintered glass filter crucibles of known weight, the precipitate being washed with 100 c.c. of dilute ammonia (5 c.c. concentrated ammonia solution per litre). The crucibles were then dried for 1 hour at 125° to eliminate water of crystallisation and weighed, dicyanodiamide nitrogen being calculated on the weight of the contents from the formula $Ni(C_2N_4H_5O)_2$.

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Inverse probability and the use of Likelihood. By R. A. FISHER, Sc.D., F.R.S., Gonville and Caius College.

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Logicians have long distinguished two modes of human reasoning, under the respective names of deductive and inductive reasoning. In deductive reasoning we attempt to argue from a hypothesis to its necessary consequences, which may be verifiable by observation; that is, to argue from the general to the particular. In inductive reasoning we attempt to argue from the particular, which is typically a body of observational material, to the general, which is typically a theory applicable to future experience. In statistical language we are attempting to argue from the sample to the population, from which it was drawn. Since recent statistical work has shown that this type of argument can be carried out with exactitude in a usefully large class of cases (**2, 3**), by means of conceptions somewhat different from those of the classical theory of probability, it may be useful briefly to restate the logical and mathematical distinctions which have to be drawn.

The mathematical work on inverse probability of the eighteenth and nineteenth centuries, beginning with Bayes' *Essay on the doctrine of chances* (**4**) in 1763, has made it perfectly clear that, if we can assume that our unknown population has been chosen at random from a super-population, or population of populations, the characteristics of which can be completely specified from *a priori* knowledge, then the statement of our inferences from the sample to the population can be put into a purely deductive form, and expressed in terms of mathematical probability. This is hardly surprising, since our data now supply us with precise information as to the generality of the populations of the kind under discussion, and we are thus in a position from the first to approach the problem deductively. Mathematicians have, however, often been tempted to apply the procedure, appropriate to this rather special case, to types of problem in which our *a priori* knowledge is certainly not of the definite kind postulated; partly perhaps because they had been trained in a great tradition of exact deductive inference, but were without example or precedent in the exact use of inductive processes; and partly because of a very remarkable feature of the mathematics, which early attracted attention, namely that as the observational material is made more and more ample, uncertainty, with respect to our *a priori* premises, makes in our result less and less difference.

In the notation used by Mr J. B. S. Haldane (1) in a recent note in these *Proceedings*, if x is an unknown probability, that is the unknown fraction of the population from which our observations are drawn, which is characterised by some observational peculiarity, and if it is known *a priori* that our population has been chosen at random from a super-population in which the frequency with which x lies in the range dx is given by the known frequency element

$$f(x)\,dx \equiv e^{\phi(x)}\,dx,$$

then the joint probability that a population shall have been chosen in the range dx, and that of n observations drawn from that population a shall be of specified kind, will be

$$\frac{n!}{a!\,(n-a)!}\,x^a(1-x)^{n-a}f(x)\,dx.$$

The numerical coefficient, which is independent of the unknown x, may be ignored in further discussion. The remainder may be interpreted as proportional to the frequency, when the data have in fact given a successes out of n, with which the unknown probability will have fallen in the range dx. Knowing the frequency distribution of x we could, of course, calculate its mean value, its median—that value which would be exceeded in 50 trials out of 100—or any other characteristic that might be required, and the fact with which we are here concerned is that, of the two factors of which our frequency element is composed, that which is contributed by, and may be calculated from, our observations, becomes, as the sample is increased, more and more influential, while the factor $f(x)\,dx$, contributed by our *a priori* knowledge, becomes less and less influential in determining these quantities, so that, subject to the very broad reservation that $f(x)$ shall be non-vanishing and differentiable at that value of x towards which a/n tends, we may say that our conclusions tend to be the same, as the abundance of our data is increased without limit, whatever the particular form of our *a priori* information.

We have of course no such assurance of the harmlessness of erroneous *a priori* assumptions, when our observations are finite in number, as is invariably the case in practice. Nevertheless, the fact under discussion has been used to justify the procedure of assigning an arbitrary function, such as $f(x)=1$, to the *a priori* distribution, in cases where it is, in reality, unknown; on the ground that such errors as we introduce in doing so, since they tend to vanish with increasingly abundant data, will not infect our conclusions with a greater uncertainty than that to which, as based on finite material, they are inevitably prone. The obvious objection to this line of argument is that, if the function $f(x)$ is in reality irrelevant to our conclusions, it should have no place in our reasoning; and that if the form of our reasoning requires its introduction, the fault lies with our adoption of this form of reasoning. The conclusion to be drawn from the decreasing importance of our *a priori* information is not the trivial one that, by introducing false *a priori* data, we may quite possibly not be led far astray, but, rather, that it indicates the fundamentally different position that conclusions can be drawn from the data alone, and that, if the questions we ask seem to require knowledge prior to these, it is because, through thinking only in terms of mathematical probability, and of the deductive processes appropriate to it, we have been asking somewhat the wrong questions. The assumption which has misled us is that, because many statements of uncertain inference can be made with precision in terms of mathematical probability, therefore this same concept is competent for the exact specification of all forms of uncertain inference of which the human mind is capable.

In cases therefore in which we allow our total ignorance of the super-population from which our population might be supposed to have been drawn, or in which, having some vague knowledge, we are unwilling to admit it as the basis of precise mathematical inference, the information supplied by the sample, as the basis for a purely inductive process of reasoning, by which the properties of the population are to be inferred, is summed up in the factor

$$x^a (1-x)^{n-a}.$$

This factor is a function of x, and not a differential increment of such a function. It is not a probability and does not obey the laws of probability. It can, however, be shown to provide, not only in the estimation of a probability, but in the whole field of statistical estimation, as satisfactory a measure of "degree of rational belief" as a probability could do. For this reason I have termed it, or some arbitrary multiple of it, the *likelihood*, based on the information supplied by the sample, of any particular value of x. Obviously the claim that the likelihood possesses these properties, and provides a rational basis for exact inference, can only be made in the light of a theory of estimation applicable to finite samples. In (2) I have developed such a theory, and have demonstrated that the most likely value of x, that is the particular estimate found by the method of maximum likelihood, possesses uniquely those sampling properties which are required of a satisfactory estimate.

For the details of this work the reader must be referred to the paper in question. At the present it will suffice to mention that when a *sufficient* statistic exists, that is one which in itself supplies the whole of the information contained in the sample, that statistic is the solution of the equation of maximum likelihood: that when no sufficient statistic exists, the solution of the equation of maximum likelihood is *efficient* in the sense that the quantity of information lost tends in large samples to a zero fraction of the whole, and that this solution contains more information than other efficient statistics. Further, setting aside, for simplicity of statement, cases involving discontinuities, the limiting value of the amount of information lost may be reduced to zero by calculating, again from the likelihood, a second statistic ancillary to the primary estimate, and indeed may be reduced to a zero of any order by a series of such ancillary statistics. These latter properties are of interest in showing that, though the primary problem of estimation is solved by one feature only, namely the *maximum* of the likelihood, yet when we press for the fullest information obtainable from a finite body of data, it is the whole course of the function which has to be used.

The outline above will show sufficiently clearly that a correction is needed on one or two points on which Mr Haldane alludes to this work. On page 60 in reference to the method of maximum likelihood he says:

"In this case Fisher showed (subject to the tacit assumption that all values of x in the neighbourhood of the optimal value are equally probable *a priori*) that the probability density of x is given by

$$dp = \frac{1}{(2\pi)^{\frac{1}{2}}\sigma} e^{-\frac{(x-\bar{x})^2}{2\sigma^2}} dx,$$

where $\bar{x}$, the optimal value, is the root of

$$L'(x) = 0 \text{ and } \sigma^{-2} = -L''(\bar{x})."$$

I had hoped that it should be clear that my work was based not on the tacit assumption of equal *a priori* probability, but upon the explicit rejection of this assumption. A closer reading of the passage in its context shows, however, that the theorem I am credited with does not belong to me at all; for Haldane's distribution is that of the unknown parameter x, and the theorem to which he evidently alludes deals with the sampling distribution about this true value of the optimal estimates, which we should obtain from different samples.

The text continues as follows:

"Fisher defines the likelihood of x as a quantity proportional to $e^{L(x)}$. This is a convenience of statement, but the introduction of the *a priori* probability density $f(x)$ allows the deduction of Fisher's results without introducing concepts other than those found in the theory of direct probability. The method of maximum likelihood in its complete form is only applicable where σ is somewhat smaller than the difference between $\bar{x}$ and the upper or lower limits which it can possibly attain, and where the graph of

$L(x)$ can be adequately fitted by a parabola in the neighbourhood of $\bar{x}$."

I suppose I need not protest against the comprehensive phrase "Fisher's results", for, as far as I can judge, the rather vague conclusion that the maximum likelihood estimate will be pretty near the middle of any reasonable inverse probability distribution. The phrase "the method of maximum likelihood in its complete form" seems, if I read it aright, to refer to Haldane's use of the method of inverse probability, and it would be less misleading to students if he had used some such term. I imagine that it is this same method which is ascribed to me lower in the page:

"Hence it is a sufficient condition for the validity of Fisher's theory that $[\phi'(x)]^2$ should be small compared with $-L''(\bar{x})$ in the neighbourhood of $x = \bar{x}$", and I must insist that, in so far as I have been guilty of a theory, it is entirely independent of the properties of ϕ, and its derivatives, and that the principal point of it lies in this independence.

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(1) J. B. S. HALDANE, "A note on inverse probability", *Proc. Cambridge Phil. Soc.*, 28 (1932), 55-61.

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(3) R. A. FISHER, "Inverse probability", *Proc. Cambridge Phil. Soc.*, 26 (1930), 528-535.

(4) T. BAYES, "An essay towards solving a problem in the doctrine of chances", *Phil. Trans.*, 53 (1763), 370-418.

PRECISION RECORDS IN HORTICULTURE.

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INTRODUCTION.

The present study is based on observations made on apple trees planted in connection with the Ministry of Agriculture's Horticultural-Meteorological Scheme. This scheme had as its object the study of the relation between weather and the growth of horticultural crops.

A meteorological station, where one did not already exist, was set up in every station participating in the scheme and observations on apples, plums, black currants and peas were made in each place. The observations were started in 1925 and are still continuing. It was laid down that the varieties used were to be the same in each place and a standardised programme of observations was drawn up.

Here we are only concerned with apples. The original scheme laid down that four " maiden " bush trees of both Bramley's Seedling and Worcester Pearmain should be planted on Doucin (Type II.) stock. By 1928 there were actually eight stations (Gulval, Wisbech, Long Ashton, East Malling, Wye, Cambridge, Osgodby and Houghall) at which these varieties were being observed. But three stations (Gulval, East Malling and Osgodby) used temporary substitutes for the observations until the new trees were old enough to observe. In 1926 and 1927 at Gulval and in 1926 at East Malling both temporary substitutes and the new trees were observed. In these cases the new trees were used for measurements of growth (length of three principal leaders taken monthly) and the substitutes for the other observations. At Newton Abbott, Wisley, Perdiswell and Sutton Bonnington comparable trees were not observed. Thus up to 1928 no very large body of comparable data had been obtained. However, the leader measurements at East Malling, Long Ashton, Osgodby and Houghall in the years 1926, 1927 and 1928 were comparable and have been used for the study of growth rates which forms Part I. of this paper.

In addition to the leader measurements the following observations were also to be taken:

(1) Date of (*a*) First open flower.
  (*b*) Full blossom (50 per cent. of flowers in blossom).
  (*c*) Dropping of petals of last flower.

(2) Observer's note on character and duration of blooming season.

(3) Number of blossom trusses (or general observation on quantity of blossom).

(4) General observation on extent of set of fruit.

(5) Time of drop.

(6) Date when bulk of the fruit is ripe.

(7) (*a*) Crop from each tree and total crop gathered, not including June drop, with note of accidental loss.

(*b*) Total weight of droppings, not including June drop with note of accidental loss.

(8) Note on keeping quantities of fruit.

(9) Diseases and pests (character, date of appearance etc.).

(10) Duration of defoliation period and colour of foliage during this period.

The data were not examined statistically until 1929, when it was found that many of the observations were not so precise and comparable as between different observers working in different places as to make it likely that, even if continued for many years, they would be of great value for correlation with weather phenomena.

For example, an estimate, based merely on inspection of a tree, can determine the 50 per cent. point of flowering neither accurately nor in a manner comparable between different observers in different places.

It appeared that the difficulty could be met by sampling observations of a precise type, accordingly a "*precision-record*" experiment for flowering was carried out on the eight apple trees on the "Horticultural Scheme" plot at East Malling.

This experiment, which was principally concerned with time of flowering, is discussed in Part II. of this paper. Although the determination of dates of flowering in a precise and comparable manner was its main object, it is to be noted that the same principles could be used to get precise and comparable observations on many of the other phenomena which the scheme was designed to study, for example fruit set, June drop, and defoliation and perhaps certain diseases such as apple scab.

PART I.

ON THE GROWTH RATES OF APPLE TREES.

Since 1925 all the horticultural stations which are carrying out the Ministry of Agriculture's Horticultural Scheme, have been taking monthly measurements of three principal leaders on four trees of each of two varieties of apple-trees, Bramley's Seedling and Worcester Pearmain.

The data at four stations, Long Ashton, East Malling, Osgodby and Houghall, in the seasons 1926, 1927 and 1928 are reasonably comparable and may be used for a study of growth rates.

All the trees were three years old in 1926 and on Malling Type II. stock.

The following table shows the units in which the measurements were taken and the degree of accuracy.

TABLE I.

	Long Ashton.	East Malling.	Osgodby.	Houghall.
1926	nearest ½ centimetre.	nearest ½ centimetre.	nearest ¼ in.	*May* nearest ¼ in. *June* nearest ½ in. rest nearest 1 in.
1927	,,	,,	nearest ½ in.	nearest 1 in.
1928	nearest $\frac{1}{10}$ centimetre.	,,	,,	June nearest ½ in. rest nearest 1 in.

Where measurements were made in inches, they were afterwards converted by the Ministry into centimetres, in 1926 to the nearest half-centimetre and in 1927 and 1928 to the nearest tenth-centimetre.

It is unfortunate that a uniform method of measurement was not adopted, this will be remedied in any future work done ; measurements to the nearest centimetre would be a reasonable level of accuracy.

Using the measurements as given in centimetres, growth rates were calculated on each leader for the months of May, June, July, August and September and relative growth rates for the months of July, August and September. These growth rates were then analysed by the analysis of variance method.*

In each month we have 288 growth rates ; from three leaders on each of four trees of each of two varieties in each of four stations in each of three seasons.

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* The analysis of variance method is one which is being increasingly used by statisticians as, when appropriately handled, it is a very powerful weapon. It has been shown that when we have a series of observations which may be classified into several categories (the number of subdivisions of one category being the same for all the subdivisions of the others) the sum of the squares of the deviations of all the observations from the mean ($S(x—\bar{x})^2$) may be expressed as the sum of the number of portions. These portions can be regarded as due to differences within the individual categories and to interactions between these differences. By an interaction between two categories is meant that the differences within any one category vary as we proceed from one subdivision to another of the second category. Thus in the present instance the sum of the squares of the deviations of the 288 observations from their mean can be split up into portions due respectively to seasonal, local and varietal differences and the interactions between them. Now it can be shown that if our data were homogeneous, that is to say if there were really no seasonal, local or varietal differences at all, on dividing each of the portions into which $S(x—\bar{x})^2$ has been split up by an appropriate

We thus have 287 degrees of freedom. There are 24 groups of four trees, so that we have 23 degrees of freedom for differences between groups and, since there are 12 growth rates in each group, 264 (11 × 24) degrees of freedom within groups. These 264 may be subdivided into 72 (3 × 24) for differences between trees in the same group and 192 (96 × 2) for differences between leaders on the same tree. This follows because there are 32 trees observed in each of three seasons ($3 \times 32 = 96$) and three leaders measured on each tree. The 23 degrees of freedom between groups can of course be subdivided into portions due to seasonal, place, and varietal differences and their interactions. In fact the complete allocation of degrees of freedom is as follows:

Differences due to:	*Degrees of Freedom.*	
Season	2	
Place	3	
Variety	1	
Interaction between		
Variety and Season	2	
Variety and Place	3	
Place and Season	6	
Second Order Interaction		
Variety, Place and Season	6	
	23	
Trees in Same Group of 4	72	Estimate of Error.
Leaders on Same Tree	192	
	287	

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number known as the *degrees of freedom* we should get an estimate of the same *variance*, namely the total variance of our 288 observations. There estimates would only differ owing to experimental error or kindred sampling fluctuations.

In actual practice we find a course that on dividing say the sum of squares due to season by its *degrees of freedom* we get a larger quantity than that which arises from trees which belong to the same group of four. If this is significantly larger, and a precise test (known as the " z " test) exists for determining whether it *is* significantly larger or not, then we know that season is having a significant effect—the same applies to the other categories and to the interactions between them.

The *degrees of freedom* for any one portion of $S(x—x)^2$ can be shown to be equal to the number of *independent* squares into which that portion can be divided.

The *degrees of freedom* of the separate portions add up to the degrees of freedom corresponding to the whole, a number which is one less than the total number of observations.

In practice the analysis is performed by first assigning to each portion the right number of degrees of freedom. This fixes the form of the analysis which can then easily be performed.

The 264 degrees of freedom within groups can be used for our estimate of error.

Tables II. and III. show the results of the analyses of variance for the growth rates and relative growth rates.*

The magnitude of the variances due to season, place variety and their interactions may be compared with that due to leaders which belong to the same group of 4 trees—the degrees of freedom corresponding to these latter are the 264 which have been bracketed together under " estimate of error."

If any of the former variances are significantly larger than the latter then they show up a real effect. The significance is tested by what is known as the " *z* " test. If " *z* " $= \frac{1}{2} \log \frac{v_1}{v_2}$ where v_1, v_2, are two independent estimates of variance " *z* " will be zero if v_1 and v_2 are exactly equal, but fluctuations of sampling will in any case make it different from zero.

Now R. A. Fisher† has tabulated the 5 per cent. and 1 per cent. values of " *z* " such that fluctuations of sampling would only give rise to a large or larger values respectively, once in twenty and once in a hundred times. These values depend on n_1 and n_2 the number of degrees of freedom on which the estimates v_1 and v_2 are based. Here the less stringent 5 per cent. test has been adopted as a criterion of significance. For instance, to test the significance of variance due to season, we work out half the natural logarithm of its ratio to the variance due to error, this gives "*z*." We then enter Fisher's table with $n_1 = 2$, $n_2 = 264$ and determine the 5 per cent. value of " *z*." If this is exceeded by the value above determined, the effect of season is regarded as significant. In Tables II. and III. those portions of the variance which are not significant as judged by the 5 per cent. " *z* " test are put in brackets.

Tables IV.-XI. give the mean values and appropriate standard errors in each month, year and place for each variety.

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* The growth rate in cms. per day for any one leader is obtained by dividing the growth of the leader during the month, measured in cms., by the number of days in the month.

The relative growth rate is the proportional amount by which the growth increases in the unit of time. Thus if U_0 be the length of a leader at the beginning of a month of thirty-one days, U_1 at the end of the month, a the relative growth rate per day is given by

$$U_0 \ (1+a)^{31} = U_1$$

$$\text{or } \log_e (1+a) = \tfrac{1}{31} (\log_e U_1 - \log_e U_0)$$

or sufficiently approximately

$$a = \tfrac{1}{31} (\log_e U_1 - \log_e U_0).$$

Thus the relative growth rate can be conveniently calculated by subtracting the natural logarithms of the leader length at the beginning from that at the end of the month, and dividing by the number of days in the month. The result can then be multiplied by 100 to express it as a percentage per day.

† Statistical methods for Research Workers—Appendix.

TABLE II.

Growth Rates—Apples (cms. per day)—Analyses of Variance.

		MAY.		JUNE.		JULY.		AUGUST.		SEPTEMBER.	
Due to	D.F.	S. of S.	Mean Square.	S. of S.	Mean Square.	S. of S.	Mean Square.	S. of S.	Mean Square.	S. of S.	Mean Square.
Season	2	.088	.044	.539	.270	.013	(.007)	.720	.360	.359	.180
Place	3	3.353	1.118	2.325	.775	4.276	1.425	2.478	.826	.507	.169
Variety	1	.262	.262	.039	(.039)	.001	(.001)	.088	.088	.039	.039
Interaction between : Variety and Season ..	2	.408	.204	.231	.116	.256	.128	.182	.091	.038	(.019)
Variety and Place ..	3	.049	.016	.078	(.026)	.085	(.028)	.228	.076	.042	(.014)
Place and Season ..	6	1.115	.186	3.695	.616	1.415	.236	.731	.122	.280	.047
Second Order Interaction : Variety, Place and Season..	6	.165	.027	.326	.054	.169	(.028)	.403	.067	.076	(.013)
Trees in same group of 4 .. Leaders on same tree ..	72 192	.633 .818	.009 .004 }.005	2.660 3.596	.037 .019 }.024	5.148 4.256	.072 .022 }.036	2.749 2.888	.038 .015 }.021	.688 1.189	.010 .006 }.007
Total	287	6.891		13.489		15.619		10.467		3.218	

D.F. = Degrees of Freedom.

S. of S. = Sum of Squares.

Figures in brackets are not significant on the basis of the 5% " z " test

TABLE III.

Relative Growth Rates—Apples (% per day)—Analyses of Variance.

		JULY.		AUGUST.		SEPTEMBER.	
Due to	D.F.	S. of S.	Mean Square.	S. of S.	Mean Square.	S. of S.	Mean Square.
Season	2	.361	(.181)	2.775	1.388	1.869	.935
Place	3	108.653	36.218	12.656	4.219	2.843	.948
Variety	1	1.468	(1.468)	.118	(.118)	.040	(.040)
Interaction between : Variety and Season	2	3.472	1.736	.981	.491	.073	(.037)
Variety and Place	3	.428	(.143)	1.404	.468	.170	(.057)
Place and Season	6	35.052	5.842	10.457	1.743	2.603	.434
Second Order Interaction : Variety, Place and Season ..	6	6.300	1.050	2.688	.448	.365	(.061)
Trees in same group of 4 ..	72	48.095	.668 } .475	14.297	.119 } .138	7.020	.098 } .060
Leaders on same tree	192	77.350	.403 }	22.263	.116 }	8.789	.046 }
Total	287	281.179		67.639		23.772	

D.F. = Degrees of Freedom.

S. of S. = Sum of Squares.

Figures in brackets are not significant on the basis of the 5% " *z* " test.

The results which they show are discussed fully in the next section of the paper, but a word may be interpolated here on the use of the standard errors at the foot of the tables. The standard error is smaller the greater the number of observations on which the average is based (being inversely proportional to the square root of that number). If it is desired to compare two averages based on the same number of observations their difference may be compared with 2.8 or roughly three times the appropriate standard error given below the table.

Thus in Table IV. we find that at Long Ashton in May, 1926, the growth rate of Bramley exceeded that of Worcester by .145, the corresponding standard error is .023, three times this is .069, so that a difference of .145 may be regarded as significant. But if we wished to compare the means of the two varieties at Long Ashton in 1926 and 1927, whose difference is .070, the appropriate standard error is .016. Again the result would be significant. The reason for the smaller standard error is that twice the number of observations have gone to form the average. Other cases may be treated analogously.

If we desire to compare two averages based on different numbers of observations we must compare the difference between the averages with its standard error. The latter is obtained by taking the square root of the sum of the squares of the standard errors of the separate averages. A difference which exceeds twice this may be regarded as significant.

Thus in Table IV., to compare Worcester at Long Ashton in 1926 with the mean at that place for all three years (both varieties), we have $.180 - .162 = .018$

The standard error of this difference is $\sqrt{(.023)^2 + (0.009)^2} = .025$ and the difference is not significant.

TABLE IV.

Growth Rates. May.

(Average Values—cms. per day per Leader.)

		1926		1927		1928		All Years	
Long Ashton ..	W	.162	.235	.192	.165	.099	.141	.151	.180
	B	.307		.137		.182		.209	
East Malling ..	W	.231	.277	.555	.477	.285	.374	.357	.376
	B	.322		.400		.463		.395	
Osgodby	W	.009	.082	.224	.238	.165	.234	.133	.185
	B	.154		.252		.303		.236	
Houghall	W	.118	.128	.000	.000	.053	.105	.057	.077
	B	.137		.000		.156		.098	
All Places	W	.130	.180	.243	.220	.151	.213	.175	.205
	B	.230		.197		.276		.234	

		cms. per day.
Standard Errors. For Individual Entries	(one variety)	.023
	(mean of both varieties)	.016
Means of all Places in Individual Years	(one variety)	.012
	(both)	.008
Means of all Seasons in Individual Places	(one variety)	.013
	(both)	.009
General Means	(one variety)	.006
	(both)	.005

TABLE V.

Growth Rates. June.

(Average Values—cms. per day per Leader.)

		1926.		1927.		1928.		All Years	
Long Ashton ..	W	·293	·340	·420	·535	·179	·126	·297	·334
	B	·387		·649		·072		·369	
East Malling ..	W	·467	·437	·422	·481	·442	·441	·444	·453
	B	·408		·539		·440		·462	
Osgodby	W	·444	·422	·613	·651	·712	·666	·590	·580
	B	·399		·688		·619		·569	
Houghall	W	·590	·593	·310	·302	·275	·314	·392	·403
	B	·597		·295		·353		·415	
All Places	W	·449	·448	·441	·492	·402	·387	·431	·443
	B	·448		·543		·371		·454	

		cms. per day.
Standard Errors. For Individual Entries	(one variety)	.014
	(mean of both varieties)	.010
Means of all Places in Individual Years	(one variety)	.007
	(both)	.005
Means of all Seasons in Individual Places	(one variety)	.008
	(both)	.006
General Means	(one variety)	.004
	(both)	.003

TABLE VI.

Growth Rates. July.

(Average Values—cms. per day per Leader.)

		1926.		1927.		1928.		All Years.	
Long Ashton ..	W	.227	.286	.093	.137	.054	.042	.125	.155
	B	.344		.180		.030		.185	
East Malling ..	W	.137	.202	.395	.327	.390	.346	.307	.292
	B	.268		.260		.302		.277	
Osgodby	W	.381	.396	.380	.392	.601	.549	.454	.446
	B	.411		.403		.497		.437	
Houghall	W	.462	.471	.423	.448	.463	.427	.449	.448
	B	.480		.472		.390		.447	
All Places	W	.302	.339	.323	.326	.377	.341	.334	.335
	B	.376		.329		.305		.337	

		cms. per day.
Standard Errors. For Individual Entries	(one variety)	.054
	(mean of both varieties)	.038
Means of all Places in Individual Years	(one variety)	.027
	(both)	.019
Means of all Seasons in Individual Places	(one variety)	.031
	(both)	.022
General Means	(one variety)	.016
	(both)	.011

TABLE VII.

Relative Growth Rates. (% per day.) July.

		1926.		1927.		1928.		All Years.	
Long Ashton ..	W	1.007	.973	.532	.540	.636	.481	.725	.665
	B	.939		.547		.327		.604	
East Malling ..	W	.520	.703	1.078	.905	1.339	1.142	.979	.917
	B	.887		.732		.945		.855	
Osgodby	W	2.350	2.106	1.252	1.222	1.717	1.589	1.773	1.639
	B	1.862		1.192		1.460		1.505	
Houghall	W	1.521	1.505	2.418	2.757	2.828	2.420	2.256	2.227
	B	1.488		3.095		2.011		2.198	
All Places	W	1.350	1.320	1.320	1.320	1.630	1.408	1.433	1.362
	B	1.294		1.392		1.186		1.291	

		%
Standard Errors. For Individual Entries	(one variety)	.199
	(mean of both varieties)	.141
Means of all Places in Individual Years	(one variety)	.099
	(both)	.070
Means of all Seasons in Individual Places	(one variety)	.114
	(both)	.081
General Means	(one variety)	.057
	(both)	.041

TABLE VIII.

Growth Rates. August.

(Average Values—cms. per day per Leader.)

		1926.		1927.		1928.		All Years.	
Long Ashton ..	W B	.016 .170	.093	.043 .114	.079	.032 .027	.030	.030 .104	.067
East Malling ..	W B	.093 .061	.077	.182 .177	.179	.010 .101	.055	.095 .113	.104
Osgodby	W B	.285 .572	.428	.338 .378	.358	.157 .122	.140	.260 .357	.309
Houghall	W B	.150 .157	.153	.320 .156	.238	.157 .168	.163	.209 .160	.185
All Places	W B	.136 .240	.188	.221 .206	.214	.089 .105	.097	.149 .184	.166

		cms. per day.
Standard Errors. For Individual Entries	(one variety)	.042
	(mean of both varieties)	.030
Means of all Places in Individual Years	(one variety)	.021
	(both)	.015
Means of all Seasons in Individual Places	(one variety)	.024
	(both)	.017
General Means	(one variety)	.012
	(both)	.009

TABLE IX.

Relative Growth Rates. (% per day.) August.

		1926		1927.		1928.		All Years.	
Long Ashton ..	W	.062	.222	.155	.251	.383	.335	.200	.269
	B	.382		.347		.286		.338	
East Malling ..	W	.332	.262	.409	.404	.032	.146	.258	.271
	B	.192		.398		.259		.283	
Osgodby	W	.944	1.231	.775	.783	.317	.290	.679	.768
	B	1.518		.792		.264		.858	
Houghall	W	.367	.376	1.023	.744	.537	.537	.642	.552
	B	.385		.465		.536		.462	
All Places	W	.426	.523	.591	.546	.317	.327	.445	.465
	B	.619		.501		.336		.485	

		%
Standard Errors For Individual Entries	(one variety)	.107
	(mean of both varieties)	.076
Means of all Places in Individual Years	(one variety)	.054
	(both)	.038
Means of all Seasons in Individual Places	(one variety)	.062
	(both)	044
General Means	(one variety) . ..	.031
	(both)	.022

TABLE X.

Growth Rates. September.

(Average Values—cms. per day per Leader.)

		1926		1927.		1928.		All Years	
Long Ashton ..	W	.034	.079	.037	.060	.009	.011	.027	.050
	B	.124		.083		.012		.073	
East Malling ..	W	.099	.080	.000	.000	.000	.000	.033	.027
	B	.061		.000		.000		.020	
Osgodby	W	.166	.155	.137	.204	.007	.018	.103	.126
	B	.143		.271		.030		.148	
Houghall	W	.112	.115	.119	.141	.092	.089	.108	.115
	B	.117		.162		.085		.121	
All Places	W	.103	.107	.073	.101	.027	.029	.068	.079
	B	.111		.129		.032		.091	

		cms. per day.
Standard Errors. For Individual Entries	(one variety)	.024
	(mean of both varieties)	.017
Means of all Places in Individual Years	(one variety)	.012
	(both)	.008
Means of all Seasons in Individual Places	(one variety)	.014
	(both)	.010
General Means	(one variety)	.007
	(both)	.005

TABLE XI.

Relative Growth Rates. (% per day.) September.

		1926.		1927.		1928.		All Years.	
Long Ashton ..	W	.118	.194	.110	.158	.082	.100	.103	.151
	B	.269		.207		.118		.198	
East Malling ..	W	.322	.273	.000	.000	.000	.000	.107	.091
	B	.224		.000		.000		.075	
Osgodby	W	.432	.367	.272	.374	.019	.042	.241	.261
	B	.302		.475		.065		.281	
Houghall	W	.219	.244	.564	.549	.273	.254	.352	.349
	B	.268		.534		.234		.345	
All Places	W	.273	.269	.237	.270	.094	.099	.201	.213
	B	.266		.304		.104		.225	

		%
Standard Errors. For Individual Entries	(one variety)	.071
	(mean of both varieties)	.050
Means of all Places in Individual Years	(one variety)	.035
	(both)	.025
Means of all Seasons in Individual Places	(one variety)	.041
	(both)	.029
General Means	(one variety)	.020
	(both)	.014

(i) *Place and Seasonal Differences and their Interactions.*

We can get the clearest view of the meaning of our results, if in each year and month we arrange the four places in order of their growth rates. In the following table E.M. stands for East Malling, L.A. for Long Ashton, O. for Osgodby and H. for Houghall. A mean value is put in the same line as its neighbour when not significantly different from it.

TABLE XII.

Places in Descending Order of Growth Rates.

May. Growth Rates.

1926.	1927.	1928.	All Years.
E.M., L.A.	E.M.	E.M.	E.M.
H	O	O	O, L.A.
O	L.A.	L.A.	H
	H	H	

June. Growth Rates.

1926.	1927.	1928.	All Years.
H	O	O	O
E.M., O	L.A.	E.M.	E.M.
L.A.	E.M.	H	H
	H	L.A.	L.A.

July. Growth Rates.

1926.	1927.	1928.	All Years.
H, O	H, O, E.M.	O	H, O
L.A., E.M.	L.A.	H, E.M.	E.M.
		L.A.	L.A.

July. Relative Growth Rates.

1926.	1927.	1928.	All Years.
O	H	H	H
H	O	O	O
L.A., E.M.	E.M., L.A.	E.M.	E.M.
		L.A.	L.A.

August. Growth Rates.

1926.	1927.	1928.	All Years.
O	O	H, O	O
H, L.A., E.M.	H, E.M.	E.M., L.A.	H
	L.A.		E.M., L.A.

August. Relative Growth Rates.

1926.	1927.	1928.	All Years.
O H, E.M., L.A.	O, H E.M., L.A.	H, L.A., O, E.M.	O H E.M., L.A.

September. Growth Rates.

1926.	1927.	1928.	All Years.
O, H, E.M., L.A.	O H L.A. E.M.	H O, L.A., E.M.	O, H L.A., E.M.

September. Relative Growth Rates.

1926.	1927.	1928.	All Years.
O, E.M., H, L.A.	H O L.A. E.M.	H L.A., O, E M.	H O L.A., E.M.

Considering first the general order as exhibited in the " All Years " column, we see that in May East Malling shows the greatest growth, as we might expect from its geographical position, Osgodby in Yorkshire and Long Ashton in Somerset are somewhat surprisingly on a level, while Houghall (Durham) lags behind. In June Osgodby has risen to top place, while Long Ashton has sunk to the bottom. In July, whether one looks at growth rates or relative growth rates, Houghall and Osgodby occupy the top position, while Long Ashton is still at the bottom. Thus the two northern stations seem to be making up for the time lost earlier in the season. In August the position of the two northern stations are reversed, otherwise the same order is maintained. In September, apart from the reversal of the two northern stations in the order of relative growth rates, the same position is maintained. It is to be noted that Long Ashton throughout occupies a lower position than one might expect.

A study of the order in the individual seasons brings out the meaning of the significant interaction between variety and place which may be noted in the analysis of variance.

The order of places in respect of the magnitude of the growth rates *does* change significantly from season to season. On the whole it appears that 1927 and 1928 were similar in their effect on this order while 1926 was relatively anomalous. The difference between 1926 and the other two seasons is most marked in May. In May, 1926, Long Ashton headed the list with East Malling; Houghall was second and Osgodby third.

As regards direct seasonal effect, in the following tables the seasons are shown in descending order of growth rates. Where one year is not significantly different from its neighbour it appears in the same line.

TABLE XIII.

Seasons in Descending Order of Growth Rates.

	Growth Rates.	Relative Growth Rates
May	1927, 1928 1926	
June	1927 1926 1928	
July	1928, 1926, 1927	1928, 1927, 1926
August	1927, 1926 1928	1927, 1926 1928
September ..	1926, 1927 1928	1927, 1926 1928

It appears that in May there was significantly less growth in 1926 than in the other two years, in June significantly less in 1926 than in 1927 and still less in 1928, in July no significant differences, while in August and September there was significantly less growth in 1928 than in the other two years.

(ii) *Direct Varietal Differences.*

Taking the average figures of all years and places, the following table shows which has the more growth, Bramley or Worcester.

TABLE XIV.

Bramley and Worcester Compared.

	Growth Rates.	Relative Growth Rates
May	B > W	
June	B > W	
July	No difference	No difference
August	B > W	No difference
September ..	B > W	No difference

Thus while there is no difference in relative growth rate, Bramley on the average has the greater absolute amount of growth.

(iii) *Interaction of Season and Variety.*

Nevertheless this is not true in all seasons, as may be gathered from the interaction between season and variety in the analysis of variance, which is significant except in September.

The meaning of this is brought out in the following table.

TABLE XV.

Bramley and Worcester Compared in Different Seasons.

(Average for all places.)

		Growth Rates.	Relative Growth Rates.
May ..	1926 1927 1928	B > W W > B B > W	
June ..	1926 1927 1928	No difference B > W W > B	
July ..	1926	B > W (just below significance line)	No difference
	1927	No difference	No difference
	1928	W > B (just below significance line)	W > B
August ..	1926 1927 1928	B > W W > B No difference	B > W No difference No difference
September	1926 1927 1928	B > W B > W No difference	No difference No difference No difference

Thus we can see that 1926 was more favourable to Bramley and less favourable to Worcester than 1927 or 1928. We have already seen that 1926 was also relatively anomalous in its effect on the order of stations in respect of growth.

(iv) *Interaction of Variety and Place and the Second Order Interaction—Variety, Place and Season.*

Reference to the Analyses of Variance shows that interactions between variety and place were significant for growth rates in May and August, and for relative growth rates in August only. The second order interaction was significant for growth rates in May, June and August and for relative growth rates in July and August. The meaning of this is brought out in the following table, which compares growth of Bramley and Worcester in all years, and in each year separately at each place:

TABLE XVI.

Bramley and Worcester Compared in Different Seasons and Places.

May. Growth Rates.

	All Years.	1926.	1927.	1928
Long Ashton ..	B > W	B > W	No difference	B > W
East Malling	B > W	B > W	W > B	B > W
Osgodby	B > W	B > W	No difference	B > W
Houghall	B > W	No difference	No difference	B > W

June. Growth Rates.

	All Years.	1926.	1927	1928.
Long Ashton	B > W	B > W	B > W	W > B
East Malling ..	No difference	W > B	B > W	No difference
Osgodby	No difference	W > B	B > W	W > B
Houghall	B > W	No difference	No difference	B > W

July. Growth Rates.

	All Years.	1926.	1927.	1928.
Long Ashton	No difference	No difference	No difference	No difference
East Malling	No difference	No difference	No difference	No difference
Osgodby	No difference	No difference	No difference	No difference
Houghall	No difference	No difference	No difference	No difference

July. Relative Growth Rates.

	All Years.	1926.	1927.	1928.
Long Ashton	No difference	No difference	No difference	No difference
East Malling	No difference	No difference	No difference	No difference
Osgodby	No difference	No difference	No difference	No difference
Houghall	No difference	No difference	B > W	W > B

August. Growth Rates.

	All Years.	1926.	1927.	1928.
Long Ashton	B > W	B > W	No difference	No difference
East Malling	No difference	No difference	No difference	No difference
Osgodby	B > W	B > W	No difference	No difference
Houghall	No difference	No difference	No difference	No difference

August. Relative Growth Rates.

	All Years.	1926.	1927.	1928.
Long Ashton	No difference	B > W	No difference	No difference
East Malling	No difference	No difference	No difference	No difference
Osgodby	B > W	B > W	No difference	No difference
Houghall	W > B	No difference	W > B	No difference

September. Growth Rates.

	All Years.	1926.	1927.	1928.
Long Ashton	B > W	B > W	No difference	No difference
East Malling	No difference	No difference	No difference	No difference
Osgodby	B > W	No difference	B > W	No difference
Houghall	No difference	No difference	No difference	No difference

September. Relative Growth Rates.

	All Years.	1926.	1927.	1928.
Long Ashton ..	No difference	No difference	No difference	No difference
East Malling	No difference	No difference	No difference	No difference
Osgodby	No difference	No difference	B > W	No difference
Houghall	No difference	No difference	No difference	W > B

First as regards interaction between variety and place, turning to the " All Years " column, we see that, in May, Bramley always shows more growth than Worcester. As the interaction is nevertheless significant, the reason must be found in the *varying amounts* by which Bramley's exceed Worcester in the different places. Reference to Table IV. shows that at Long Ashton this amount is .06 cms. per day ; .10 at Osgodby and .04 at East Malling and Houghall.

The other significant case is in August, both for growth-rates and relative growth-rates. Here we find that while the absolute growth of Bramley is greater than that of Worcester at Long Ashton and Osgodby, there is no difference at East Malling and Houghall. In relative growth, however, there is no difference at Long Ashton and East Malling ; Bramley exceeds Worcester at Osgodby and the reverse is true at Houghall.

The significant second order interaction shows us that the relative behaviour of Bramley and Worcester in the four places varies from season to season. In May, for instance, whereas in 1928 Bramley showed more growth than Worcester in all four places, in 1926 this was only true in three ; and in 1927 Worcester showed more growth than Bramley at East Malling and in the remaining places there was no difference. In June there was again significant differences in relative behaviour, but of a different nature, in August two seasons show no significant differences at all, while in 1926 Bramley showed more growth than Worcester in two stations (Long Ashton and Osgodby).

Turning to relative growth rates in July, we find no differences except in one station (Houghall), in only two seasons (1927 and 1928). In the former case Bramley beats Worcester, in the latter the reverse is true. In August in 1926 we find Bramley beating Worcester in two stations (Long Ashton and Osgodby) and no difference in the other two ; in 1927 there is only a difference at one station (Houghall), this time in favour of Worcester ; in 1928 there are no differences.

The cases where the interactions are not significant may be safely disregarded here.

SUMMARY.

(*a*) Over the period as a whole we find:

(i) East Malling (Kent) gets growth started first showing the greatest growth rates in May, but, as one might expect, loses this advantage later in the season.

(ii) Long Ashton (Somerset) shows rather low growth rate over the whole season.

(iii) Osgodby (Yorks) gets started rather early considering its geographical position, actually equalling Long Ashton in May.

(iv) Houghall (Durham) makes up for its late start by having the greatest growth rates in July.

(v) The absolute amount of growth in Bramley is greater than in Worcester, but there is no difference in the relative growth rates.

(vi) In the May and August growth rates, and in the August relative growth rate, there are significant interactions between variety and place. The advantage Bramley has over Worcester is greater at Long Ashton and Osgodby than at East Malling and Houghall.

(*b*) When we consider seasonal effects we find:

(i) In May there was significantly less growth in 1926 than in the other two years; in June significantly less in 1926 than in 1927 and still less in 1928; in July there were no significant differences, while in August and September there was significantly less growth in 1928 than in the other two years.

(ii) The order of places in respect of the magnitude of the growth rates changes significantly from season to season; 1927 and 1928 were similar in their effect, while 1926 was relatively anomalous. The difference between 1926 and the other two seasons is most marked in May. Here the order for all years is East Malling, Osgodby, Long Ashton and Houghall, while in 1926 the order is Long Ashton, East Malling, Houghall and Osgodby.

(iii) Season also has an influence on the relative behaviour of varieties, 1926 was more favourable to Bramley and less favourable to Worcester than 1927 or 1928.

(iv) The relative behaviour of Bramley and Worcester in the four places also varies significantly from season to season in the months of May, June and August for absolute growth rates, and in July and August for relative growth rates.

(v) To specify the weather factors which are responsible for these somewhat puzzling interactions with season would need data over many more years than are now available.

PART II.

ON TIME OF FLOWERING.

(i) *General Considerations.*

With the object of seeing whether the horticultural observations taken in connection with the Ministry of Agriculture's Crop Weather Scheme could not be improved by observations of a precision-record type such as have been employed successfully in the agricultural portion of the scheme, an experiment on the flowering of apple trees at East Malling was carried out in 1930. The eight trees on the plot which had been laid down in connection with the Horticultural Crop Weather Scheme of the Ministry were used. Four of the trees were Bramley's Seedling and four Worcester Pearmain, all six-year-old trees on Doucin (No. II.) stock.

Four samples of 200 cms. of wood were measured and marked off on each tree. The method of sampling employed was that now generally in use at East

Malling. The tree is approached from the four points of the compass in turn ; each time the eyes are closed and the right hand stretched out to touch a branch. From the point where a branch is touched we follow down until we reach a main branch, and then we follow the main branch up again to its tip. This may of course lead us to a different side of the tree from our starting point. From the tip of the main branch thus reached we start measuring downwards ; as soon as we come to a fork we follow the lateral up to its tip, and continue measuring from the outside inwards ; once back at the fork we continue measuring the main branch until we get to the next fork and so on. In this way any desired length of wood can be measured and we can mark the end of our measured portion with a piece of tape tied round it.

On a mature tree this is as near an approach to random sampling as is practicable ; actually on the six-year-old trees the four samples of 200 cms. of wood took up a very considerable portion of the tree, the main branch reached usually being on the same side of the tree as that touched.

On May 1st the total number of fruit buds on each tree as a whole were counted and the four samples of 200 cms. of wood were marked off.

The number of fruit buds on the sample branches were also counted. The term fruit bud is here used to mean the entity which gives rise to the truss, as distinguished from the individual blossom buds. At this time the fruit buds were just bursting and the individual blossom buds were becoming visible. On May 5th the individual blossom buds on the four samples on each of the eight trees were also counted.

Table I. shows the total number of fruit buds on each of the eight trees and also the number included in the four samples on each tree.

TABLE I.

	Tree.	Total No of Fruit Buds.	No. of Fruit Buds on Branches Sampled.
Bramley	1	108	38
,, ...	2	81	41
,, ...	3	144	51
,, ...	4	66	34
Total		399	164
Worcester	1	136	99
,, ...	2	136	116
,, ...	3	191	85
,, ...	4	223	81
Total		686	381
Grand Total ..		1,085	545

In the aggregate about half the flower trusses were therefore sampled, the proportion naturally varying from tree to tree.

The total numbers of individual flower buds on the sample branches were as follows:

TABLE II.

Number of Individual Flower Buds on Sample Branches.

Tree	Bramley.	Worcester.*
1	165	435
2	198	511
3	230	340
4	140	296
	733	1,582

Of the 733 Bramley buds only 644 ever opened, the remainder being destroyed before opening, mostly by insect pests. Of the 1,582 Worcester buds 1,546 opened.

On June 17th the fruit set on the sampled branches was counted. Twenty-two out of the thirty-two apples on the Worcesters, but only four out of the forty-eight on Bramley's were found to be attacked by saw-fly. On September 24th the mature fruits on the sampled branches were counted. The Worcesters had none and the Bramley's seventeen fruits.

The full particulars are given in Table III., which shows the percentage of fruit set and of mature fruit obtained. The percentages were calculated both per truss and per individual flower-bud number. In the latter case, had the percentage been calculated on the basis of the number of flowers which opened, the advantage of Bramley as compared with Worcester would have been even more emphasised.

The two varieties form a striking contrast. On the Worcesters there were more than double the number of flowers which were on the Bramley's, yet the latter variety set 50 per cent. more fruit. The percentage of fruit set on the Worcesters was only about a third of that of the Bramley's and finally the former yielded no fruit at all.

(ii) *Daily flowering counts.*

Starting on May 9th, daily counts were made of the number of flowers open on each of the sample branches. The number of flowers, all the petals of which had dropped, were also counted. The original data are shown in Tables

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* These are the numbers of flower buds obtained at the first count, when evidently a few were missed, because the numbers of flowers finally recorded on certain branches (as having flowered) were sometimes slightly greater than the initial number of buds. See Appendix, Tables VI.-IX.

TABLE III.

Bramley's Seedling. Four Samples of 200 cms. of wood.

				Fruit Set.			Mature Fruit.		
Tree No.	No. of Flower Buds	No. of Trusses.	Flowers per Truss.	No.	% per Truss.	% per Flower Bud.	No.	% per Truss.	% per Flower Bud.
1	165	38	4·3	18	47·4	10.9	6	15.8	3.6
2	198	41	4·8	11	26.8	5·6	3	7·3	1.5
3	230	51	4·5	11	21.6	4·8	3	5·9	1.3
4	140	34	4·1	8	23·5	5·7	5	14·7	3·6
Total	733	164	4·5	48	29·3	6.5	17	10.4	2.3

Worcester Pearmain. Four Samples of 200 cms. of wood.

				Fruit Set.			Mature Fruit.		
Tree No.	No. of Flower Buds.	No. of Trusses.	Flowers per Truss.	No.*	% per Truss.	% per Flower Bud.	No.	% per Truss.	% per Flower Bud.
1	435	99	4·4	5	5·1	1.1	*Nil.*	*Nil.*	*Nil.*
2	511	116	4·4	14	12.1	2.7			
3	340	85	4·0	8	9·4	2.4			
4	296	81	3·7	5	6.2	1·7			
Total	1,582	381	4·2	32	8.4	2.0			

* Tree 1 2 attacked by sawfly
Tree 2 11 ,, ,,
Tree 3 5 ,, ,,
Tree 4 3 ,, ,,

VI-IX. of the Appendix.[*] The complete frequency distributions both of time of opening and of time of drop were obtained from these figures.[†]

The following diagram shows the position of the trees in the plot:

N

P_4 W_2
W_4 B_2
W B_4 P_2 E
P_3 W_1
W_3 B_1
B_3 P_1

S

P_1, P_2, P_3, P_4 are plum trees not observed in this connection.

In order to investigate whether the position of the trees had any obvious influence on flowering, the frequencies were added together for the two trees of each variety on the East and on the West sides. Fig. 1 shows the cumulative frequencies, i.e., the number of flowers which had opened by given dates for W_1 and W_2 together, for W_3 and W_4 together, and similarly for the Bramley's of the East and West sides.

It will be seen that the Worcesters on the East side had more flowers than those on the West, but the reverse was true of the Bramley's.

A similar state of affairs is exhibited in Fig. 2, which shows the drop. There is even less difference between the North and South positions, so that there is no evidence here to suggest that the differences are due to the geographical position of the trees on the plot.

It has been remarked that the branches sampled on each tree were roughly in the N, S, E and W positions. Accordingly the number of flowers opening and dropping on the branches in the North, East, South and West positions were obtained for each variety. Fig. 3 shows the cumulative frequencies for opening and Fig. 4 for drop. It will be seen that there is little or no difference between branches in the West and North positions, on the Worcesters the branches on the East side had more flowers than those on the South side, and the reverse was true for the Bramley's. There is therefore no evidence that the position of branches on the tree was a factor of any importance in relation to flowering.

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[*] On May 20th on B_3 there was difficulty on the West side and on B_4 on the East side, in a few cases indistinguishing those blossoms which had flowered and were over, from those buds which had never opened but the petals of which had become shrivelled and eaten away owing to attacks of insect pests. This continued on the West side on the 21st and 22nd. Accordingly in Table IX. the numbers of flowers dropped are in certain cases marked with a star, to show that they have been estimated, where this was necessary.

[†] By the "*complete frequency distribution of time of opening*" is meant the data which show for every day in the flowering season how many flowers have opened on that day. The data may conveniently be portrayed by a diagram which represents the number of flowers opening on any day by a block whose height is proportional to that number. Frequency distribution of time of drop has a similar meaning. For examples of such diagrams see Figures 9, 10, 13 and 14.

It may be noted that each Bramley on the plot was next to a Worcester. In Figures 5 and 6 the number of flowers on adjacent pairs of trees are compared. It will be seen that there is very little difference between positions 1 and 3. But position 2 has actually done better than position 4, while 1 and 3 are intermediate. This is remarkable, considering the closeness of the two positions (2 and 4) and may be a soil effect, but we cannot say with only two trees in each position.

(iii) *Detailed study of the frequency distributions for Bramley and Worcester.*

The total frequency distributions for Bramley and Worcester (all four trees together) are shown in Figs. 7-14. Figs. 7-10 concern flowering and Figs. 11-14 concern drop. In Fig. 7 the cumulative frequencies are shown and Fig. 8 the cumulative percentage frequencies. Fig. 9 shows the actual daily frequencies and in Fig. 10 these are expressed as percentages. Figs. 11 to 14 are constructed on the same plan for the drop.

Since the main object of the experiment was to determine date of flowering and drop in a manner comparable between different observers in different places, we naturally consider this first. Date of flowering is most simply defined as the date when half the flowers have come out, and date of drop as the date when half the flowers have dropped. That is to say, we take the medians* of the frequency distributions.

We find the date of flowering was the 15th for Bramley and the 16th for Worcester, date of drop the 20th for Bramley and the 21st for Worcester.

More exactly we find:

TABLE IV.

	Bramley.				Worcester.			
	Median	Standard Error	Mean.	Standard Error.	Median	Standard Error.	Mean.	Standard Error
Flowering ..	15.50	.17	16.67	.14	16.32	.11	17.63	.09
Drop ..	20.69	.14	21.38	.11	21.70	.10	22.56	.08

We see that the precision of the experiment is more than sufficient to determine the median to the nearest day.

We also see that the mean is about a day later than the median, the distributions both of flowering and drop being perceptibly skew, rising somewhat more sharply than they fall.

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* While the median is the date by which half the flowers have come out, the mean is the average date obtained by multiplying each date by the number of flowers which came out on that date and dividing by the total number of flowers

β_2 and β_1 which are measures of the kurtosis and skewness of the distributions, have been obtained.*

These are:

TABLE V.

		Bramley.		Worcester.	
Opening	β_2	4.98	S.E .19	3.95	S.E .12
	β_1	1.61		.96	
	$\sqrt{\beta_1}$	1.27	S.E .10	.98	S.E .06
Drop ..	β_2	4.02	S.E .19	2.70	S.E .12
	β_1	.96		.31	
	$\sqrt{\beta_1}$	.98	S.E .10	.56	S.E .06

Standard Errors calculated on basis of Normality.

All the distributions are significantly leptokurtic (i.e., with higher top and longer tails than the "normal") with the exception of the distribution for "drop" in the Worcesters, which is platykurtic (i.e., with lower top and shorter tails than the "normal").

All are significantly skew, as we have already seen in comparing the medians and means.

(iv) *The weather in the flowering period.*

It is of course not possible in a single season and in a single place to determine the relation of flowering to weather, nevertheless it is interesting to examine the weather of May, 1930, to see whether any interesting effects on flowering are suggested. Daily rainfall, daily maximum wind velocity and daily sunshine are shown in Fig. 15 and the maximum and minimum temperatures in Fig. 16.

The temperature, rainfall and sunshine data are given in the Appendix Table X. and the wind data, including Beaufort numbers,† at 9, 15 and 21 hours, and the time of maximum velocity in Table XI.

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* The *skewness* is of course a measure of the departure of the curve from symmetry It is conveniently measured by the quantity $\sqrt{\beta_1}$ which is zero when the curve is symmetrical and increases as the curve becomes more and more skew. $\sqrt{\beta_1} = \frac{\mu_3}{\mu_2^{3/2}}$ where μ_3 is the mean cube of the deviation of the observed dates from the mean date and μ_2 is the mean square of these deviations. Similarly β_2 is given by $\beta_2 = \frac{\mu_4}{\mu_2^2}$. For the "normal" frequency curve, so commonly met with, $\beta_2 = 3$ and the amount by which B_2 differs from 3 is called the "*kurtosis.*" Curves with positive kurtosis or $\beta_2 > 3$ have as explained above higher tops and longer tails than the normal, for those with negative kurtosis ($\beta_2 < 3$) the reverse is the case.

† For explanation see foot of Appendix, Table XI.

There is a suggestion in Figs. 13 and 14 that the drop of Worcester flowers is more sensitive to environmental influences than the drop of Bramley's. To a lesser extent this is also suggested of the opening (Figs. 9 and 10).

It will be seen that the 14th, 15th, 16th and 17th of May were the days on which the bulk of the flowers came out. These were fine sunny or rather sunny days with maximum temperatures above 60° and minima well above 40° F. These followed a few days with rain, little sun, and lower temperatures. This suggests, as is, I suppose, a common observation, that once the buds are ready to flower, the first spell of fine warm weather will bring them out, while cool rainy weather may delay flowering for some time.

It will be seen that the 22nd-23rd May was the day on which the drop on the Worcesters reached its maximum, the maximum for the Bramley's occurring the previous day. The 22nd-23rd May covers a period from about noon on the 22nd to noon on the 23rd ; the wind velocity reached a maximum of 24 miles per hour at 7 a.m. on the 23rd and another maximum of 36 miles per hour at just after 10 a.m. on the 23rd. Both these periods come within the 22nd-23rd as defined above, thus it is easy to explain why the maximum drop on the Worcesters occurred on that day. The maximum drop on the Bramley's occurred on the 21st-22nd, on which day the maximum wind velocity was only 17 miles per hour ; thus the Bramley drop appears to be more independent of wind than that of the Worcesters, as one might expect from the less fragile nature of the flowers.

It is not easy to explain the gradual fall in numbers dropping for both varieties between the 17th and the 20th and the sudden rise in the drop on the Worcesters on the 20th-21st. Of the 17th, 18th and 19th, the 18th had the greatest maximum wind velocity, 30 miles per hour at about 5.20 p.m., otherwise the three days appear to have been about equally windy, nor do the other meteorological variates throw any obvious light on this phenomenon.

Nor is the sudden rise in the absolute and percentage numbers of Worcesters dropping on the 20th-21st easy to explain. The 20th was one of the calmest days of the month, had a relatively low maximum and high minimum temperature and only a trace of rainfall.

Thus it does not appear that this sudden increase in drop on one variety only was due to weather. It does seem on studying Figs. 13 and 14 that the drop on Worcesters is more dependent on environmental influences, but what these influences are we cannot say. If they are weather influences they must be of too subtle a nature to be revealed by an inspection of the available weather data for the month.

I am indebted to Mr. R. G. Hatton for giving me every facility in carrying out this work and to Mr. T. N. Hoblyn and numerous members of the East Malling staff for very helpful suggestions.

TABLE X.

Weather Data—May, 1930. *(East Malling.)*

Date.	Rainfall inches.	Sunshine hours.	Temperature (F.).	
			Max.	Min
May 1st	—	10.1	57	39
,, 2nd	trace	3.8	59	41
,, 3rd	.01	1.7	58	41
,, 4th	—	2.4	63	42
,, 5th	.26	6.6	64	33
,, 6th	.445	4.2	59	45
,, 7th	.005	2.7	51	43
,, 8th	—	9.4	52	38
,, 9th	.32	6.5	55	37
,, 10th	.14	5.6	55	41
,, 11th	.06	0.8	55	43
,, 12th	.09	5.6	58	45
,, 13th	.07	0.0	60	47
,, 14th	—	12.2	65	48
,, 15th	—	4.0	61	41
,, 16th	—	8.9	66	44
,, 17th	—	2.9	64	47
,, 18th	.215	10.3	61	50
,, 19th	—	6.4	59	39
,, 20th	trace	0.0	58	50
,, 21st	trace	6.6	59	47
,, 22nd	.30	1.6	57	43
,, 23rd	—	10.5	63	45
,, 24th	.19	2.9	62	47
,, 25th	.18	0.0	55	49
,, 26th	.10	1.3	62	50
,, 27th	—	10.3	68	45
,, 28th	—	13.4	71	42
,, 29th	—	11.5	71	44
,, 30th	.83	1.4	67	49
,, 31st	.23	3.9	64	54

TABLE VIII.

Bramley's Seedling. Number of Blossoms on Sample Branches—Trees B_1 and B_2.

Date.	B1.												B2.											
	N			E			S			W			N			E			S			W		
	b	f	o	b	f	o	b	f	o	b	f	o	b	f	o	b	f	o	b	f	o	b	f	o
May 5th	69			23			36			37*			47			37*			48			66		
„ 9th		—	—		—	—		—	—		—	—		—	—		—	—		—	—		1	—
„ 10th		—	—		—	—		—	—		1	—		—	—		—	—		—	—		3	—
„ 12th		—	—		—	—		3	—		2	—		1	—		—	—		—	—		8	—
„ 13th		2	—		1	—		5	—		4	—		4	—		1	—		—	—		14	—
„ 14th		5	—		1	—		7	—		9	—		7	—		1	—		3	—		19	—
„ 15th		12	—		4	—		13	—		16	—		18	—		8	—		14	—		24	—
„ 16th		16	—		5	—		19	—		20	—		28	—		10	—		29	—		31	—
„ 17th		19	—		10	—		23	—		23	—		31	—		19	—		38	—		38	3
„ 18th		22	4		11	1		18	6		18	7		34	4		23	—		39	—		29	13
„ 19th		19	7		11	3		16	10		13	13		29	9		20	5		36	3		30	13
„ 20th		19	16		7	9		10	17		14	16		26	13		22	9		34	6		24	19
„ 21st		10	25		3	13		9	18		9	21		20	21		20	11		33	13		23	23
„ 22nd		8	28		2	14		6	21		6	24		11	32		13	18		22	24		16	30
„ 23rd		1	35		—	16		3	24		3	29		5	38		7	24		4	43		6	40
„ 24th		3	35		—	16		1	26		5	29		3	40		3	28		3	44		5	42
„ 25th		2	36		—	16		2	26		8	29		1	42		1	40		1	46		4	43
„ 26th		2	36		—	16		2	26		8	29		1	42		1	40		1	46		4	43
„ 27th		1	37		—	16		2	27		8	29		—	43		—	41		—	47		4	43
„ 28th		2	37		—	16		2	28		5	33		—	43		—	41		—	47		—	47
„ 29th		1	38		1	16		2	28		—	38		—	43		—	41		—	47		—	47
„ 30th		2	38		1	16		1	29		—	38		—	43		—	41		—	47		—	47
„ 31st		—	40		—	17		—	30		—	38		—	43		—	41		—	47		—	47

* Clearly a few buds were missed at the first count.

Fruit Set (June 17th).

	B1.				B2.			
	N	E	S	W	N	E	S	W
Good	6	—	8	4	1	3	5	2

Number of Fruits—September 24th.

	3 3 on ground (2 brown rot) }	— { (origin uncertain).	3	—	—	2 1 on ground }	1 (diseased) { (origin uncertain).	1

b = initial number of buds.

f = number of flowers out.

o = number of flowers all petals of which have dropped.

S.F. = attacked by sawfly.

TABLE VI.

Worcester Pearmain. Number of Blossoms on Sample Branches—Trees W_1 and W_2.

Date.	W1.												W2.											
	N			E			S			W			N			E			S			W		
	b	f	o	b	f	o	b	f	o	b	f	o	b	f	o	b	f	o	b	f	o	b	f	o
May 5th	121			138			94			82			135			93*			125			158		
„ 9th		—	—		—	—		—	—		—	—		—	—		—	—		—	—		2	—
„ 10th		—	—		2	—		—	—		—	—		6	—		—	—		2	—		4	—
„ 12th		1	—		9	—		5	—		4	—		16	—		4	—		3	—		12	—
„ 13th		6	—		13	—		9	—		4	—		19	—		7	—		9	—		14	—
„ 14th		14	—		22	—		14	—		16	—		38	—		8	—		14	—		30	—
„ 15th		23	—		44	—		40	—		19	—		72	—		14	—		31	—		53	—
„ 16th		54	—		76	—		54	2		37	—		92	—		29	—		41	—		73	—
„ 17th		76	1		94	8		53	7		45	1		105	6		37	2		70	4		89	10
„ 18th		78	9		87	21		62	11		46	8		96	29		40	11		80	9		76	37
„ 19th		73	16		86	34		50	23		51	11		83	43		37	15		68	22		71	44
„ 20th		72	24		82	39		42	31		49	13		75	53		41	18		59	31		74	48
„ 21st		56	40		63	64		29	44		36	29		55	75		35	30		47	46		64	61
„ 22nd		44	57		32	95		26	63		27	47		30	101		36	44		30	63		54	82
„ 23rd		20	81		16	111		12	77		11	63		12	120		29	51		19	75		40	104
„ 24th		18	85		7	123		11	78		10	64		11	121		32	51		15	83		45	104
„ 25th		18	95		8	127		8	81		13	64		9	125		25	68		20	90		42	111
„ 26th		14	99		6	129		8	81		12	65		8	126		25	68		16	94		32	121
„ 27th		14	100		6	129		4	86		10	67		5	129		19	75		16	96		23	131
„ 28th		8	110		3	132		8	85		8	72		—	134		13	83		6	107		7	147
„ 29th		7	112		3	132		7	86		7	73		—	134		2	94		—	113		—	154
„ 30th		4	117		2	133		4	89		4	76		—	134		—	96		—	113		—	154
„ 31st		1	120		1	134		1	92		1	79		—	134		—	96		—	113		—	154
June 1st		—	121		—	135		—	93		—	80		—	134		—	96		—	113		—	154

* Clearly, a few buds were missed at the first count.

Fruit Set (June 17th).

	W1.				W2.			
	N	E	S	W	N	E	S	W
Good	—	—	—	3	3	—	—	—
S.F.	1	—	—	1	4	3	2	2

Number of Fruits—September 24th.

	—	—	—	—	—	—	—	—

b=initial number of buds.

f=number of flowers out.

o=number of flowers all petals of which have dropped.

S.F.=attacked by sawfly.

TABLE XI.

Records of Wind—May, 1930. (East Malling.)

Date.	Beaufort Numbers.* 9 hrs.	15 hrs.	21 hrs.	Maximum Wind Velocity (m.p h). Velocity.	Time of Occurrence †
May 1st	4	4	1	30	15.6
„ 2nd	1	1	calm	10	15.6
„ 3rd	1	calm	calm	9	12.7
„ 4th	calm	1	calm	14	18.5
„ 5th	1	2	calm	20	18.2
„ 6th	2	2	calm	21	1.0
„ 7th	3	3	calm	24	4.6
„ 8th	4	3	1	20	11.8
„ 9th	4	4	3	30	16.5
„ 10th	1	4	3	23	8.2
„ 11th	4	4	3	28	15.6
„ 12th	4	4	1	22	12.9
„ 13th	3	3	3	19	16.6 8.8
„ 14th	4	3	calm	21	11.8 12.3
„ 15th	3	4	2	20	15.0
„ 16th	3	2	1	17	9.7 16.9
„ 17th	3	4	1	26	0.8
„ 18th	4	3	2	30	17.3
„ 19th	4	4	2	20	12.8
„ 20th	1	2	calm	15	12.3
„ 21st	3	3	1	17	8.5
„ 22nd	3	4	3	24	7.0
„ 23rd	5	5	3	36	10.9
„ 24th	2	1	calm	16	2.3
„ 25th	3	1	calm	11	9–10
„ 26th	calm	calm	calm	5	18.6
„ 27th	calm	1	1	12	13.9
„ 28th	4	3	calm	20	10.7
„ 29th	3	1	calm	13	12.7
„ 30th	2	2	3	29	18.5
„ 31st	3	3	1	18	11.3

* Beaufort No 1 corresponds to 1— 3 miles per hour at 33 ft in the open.
2 „ „ 4— 7 „ „ „ „ „
3 „ „ 8—12 „ „ „ „ „
4 „ „ 13—18 „ „ „ „ „
5 „ „ 19—24 „ „ „ „ „

† The day is counted from 9 a.m. to 9 a.m for this purpose, thus 17 hrs. against the 21st means 5 p m. on the 21st, 5 hrs against the 6th means 5 a m. on the 7th 10 hrs against the 2nd means 10 a m. on the 2nd, etc

Time given to the nearest tenth of an hour, except on the 25th when there was a level trace on the chart between 9 and 10.

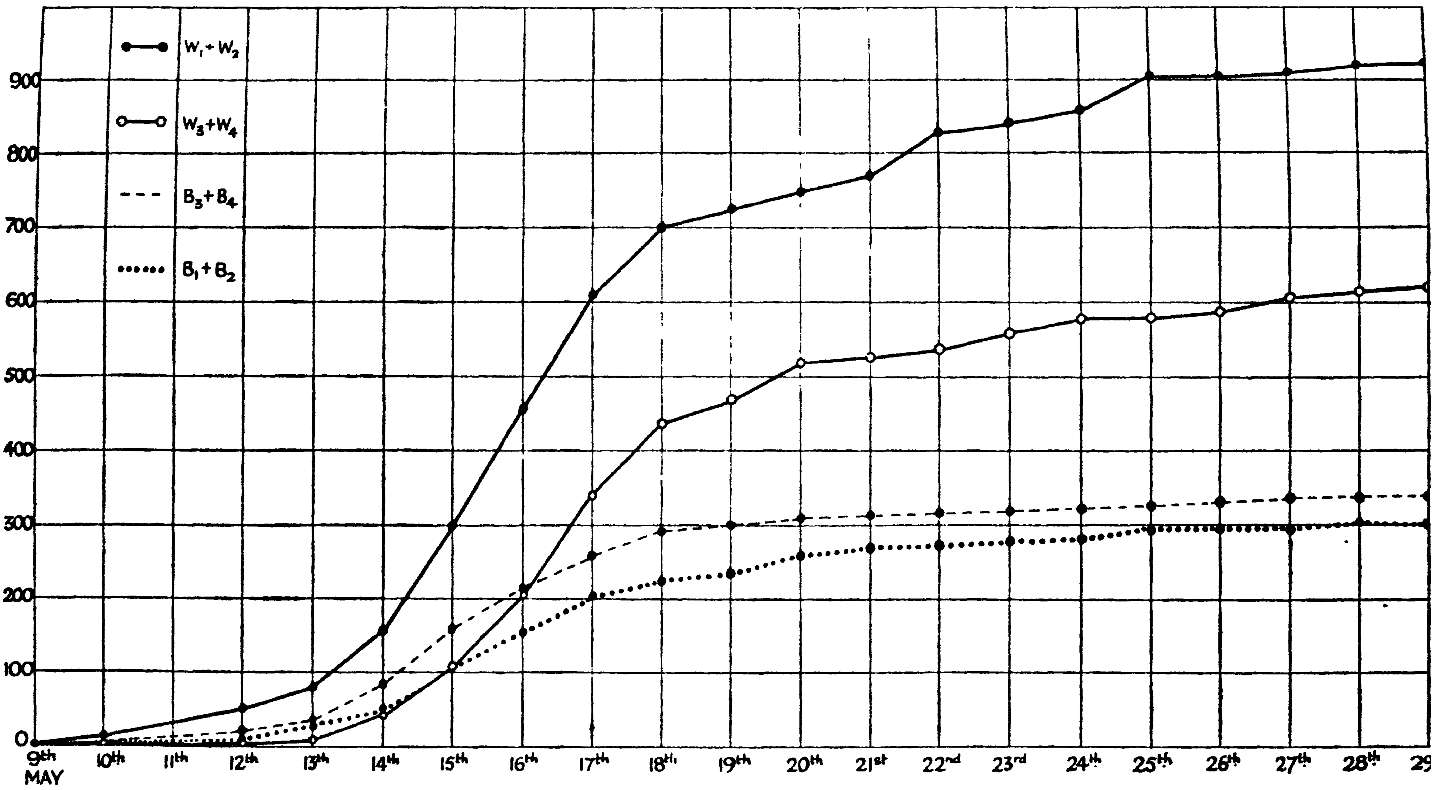


Fig. 1.

No. of Flowers which have opened by stated dates (Trees on East [1 and 2] and West [3 and 4] compared).

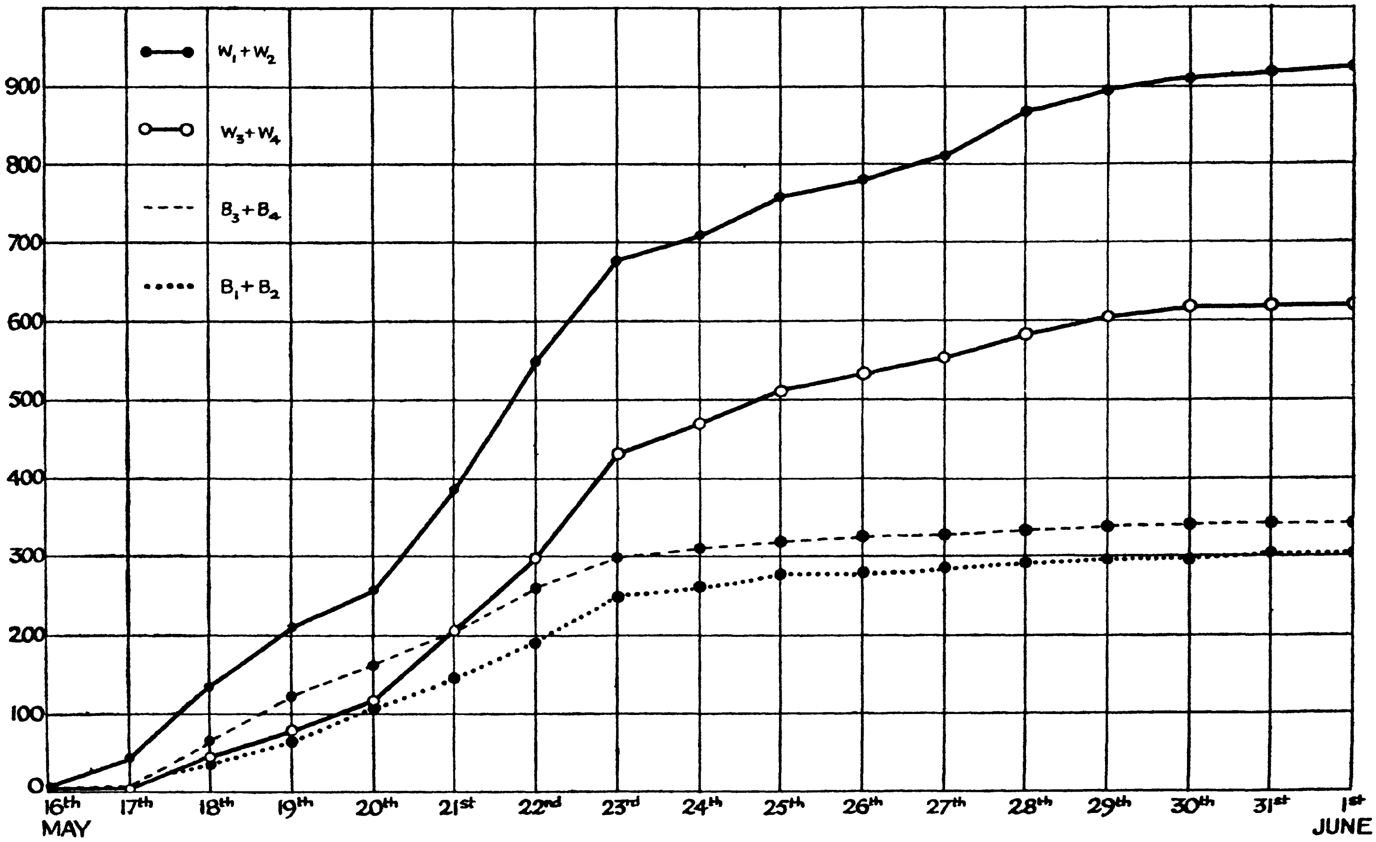


FIG 2

No of Flowers which have dropped (all petals) by stated dates (Trees on East [1 and 2] and West [3 and 4] compared).

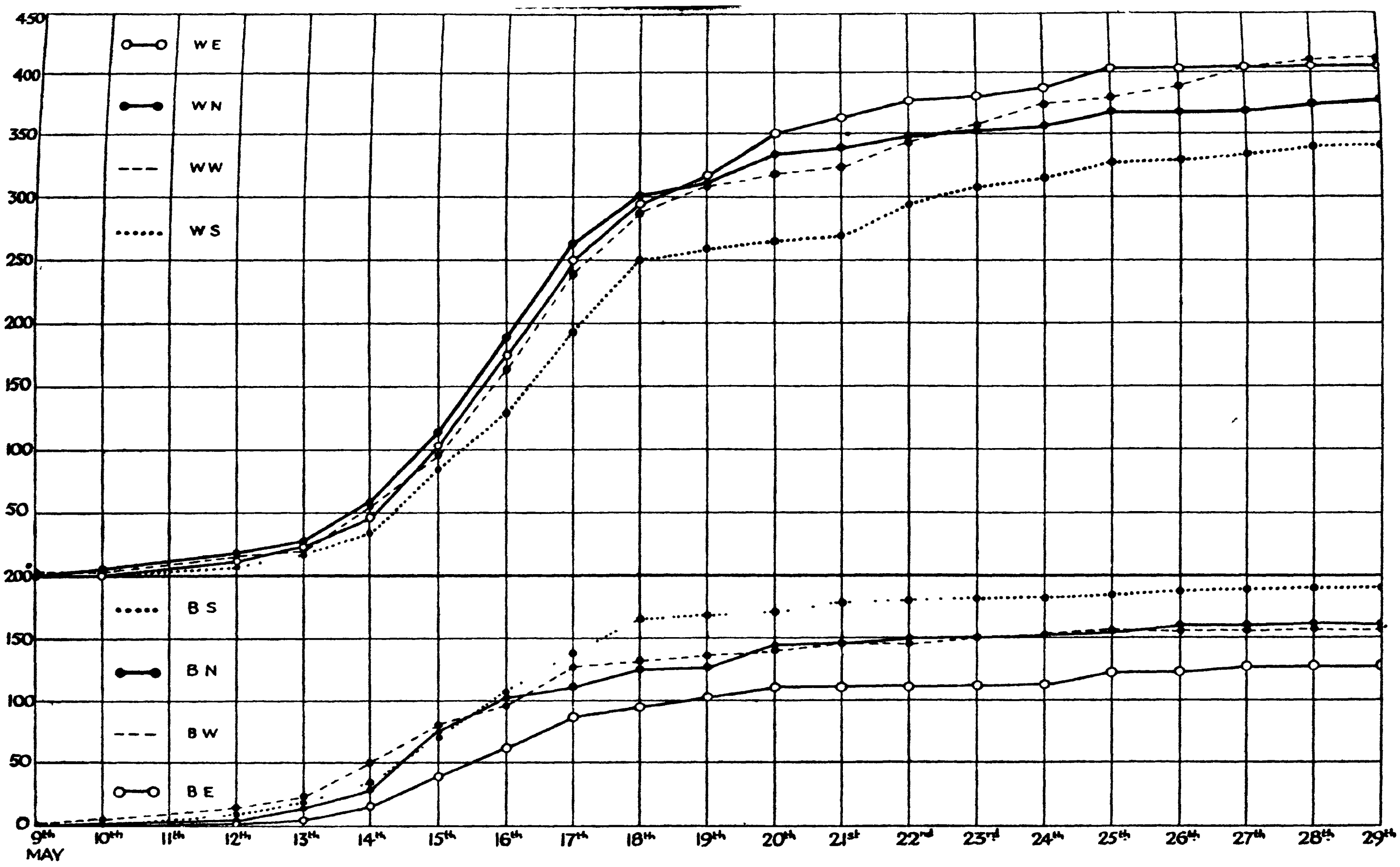
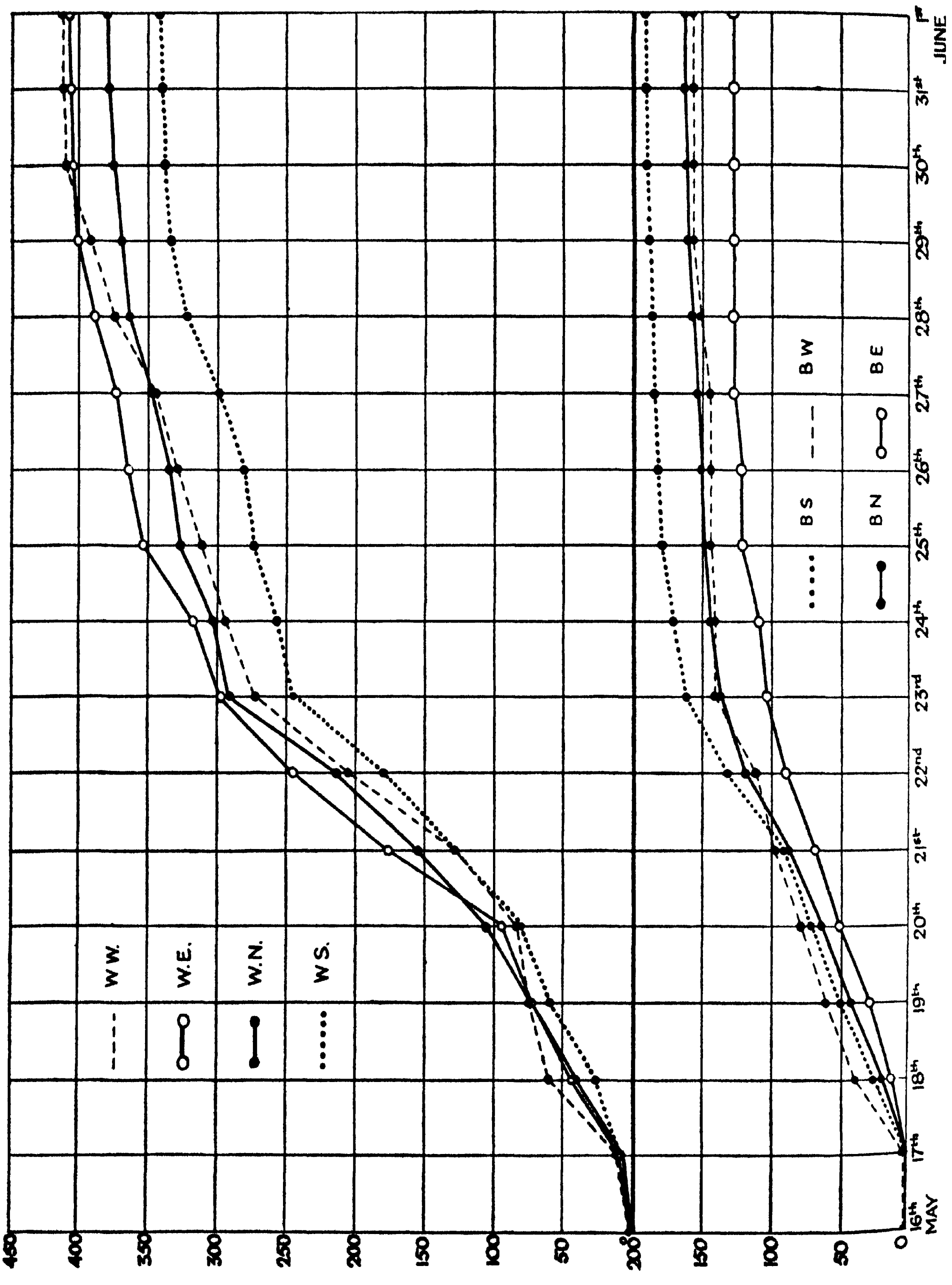


FIG. 3.

No. of Flowers which have opened by stated dates (samples in different geographical positions compared).


W.W.
W.E.
W.N.
W.S.
B S
B W
B N
B E
450
400
350
300
250
200
150
100
50
0
200
150
100
50
0
16th MAY
17th
18th
19th
20th
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22nd
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28th
29th
30th
31st
1st JUNE

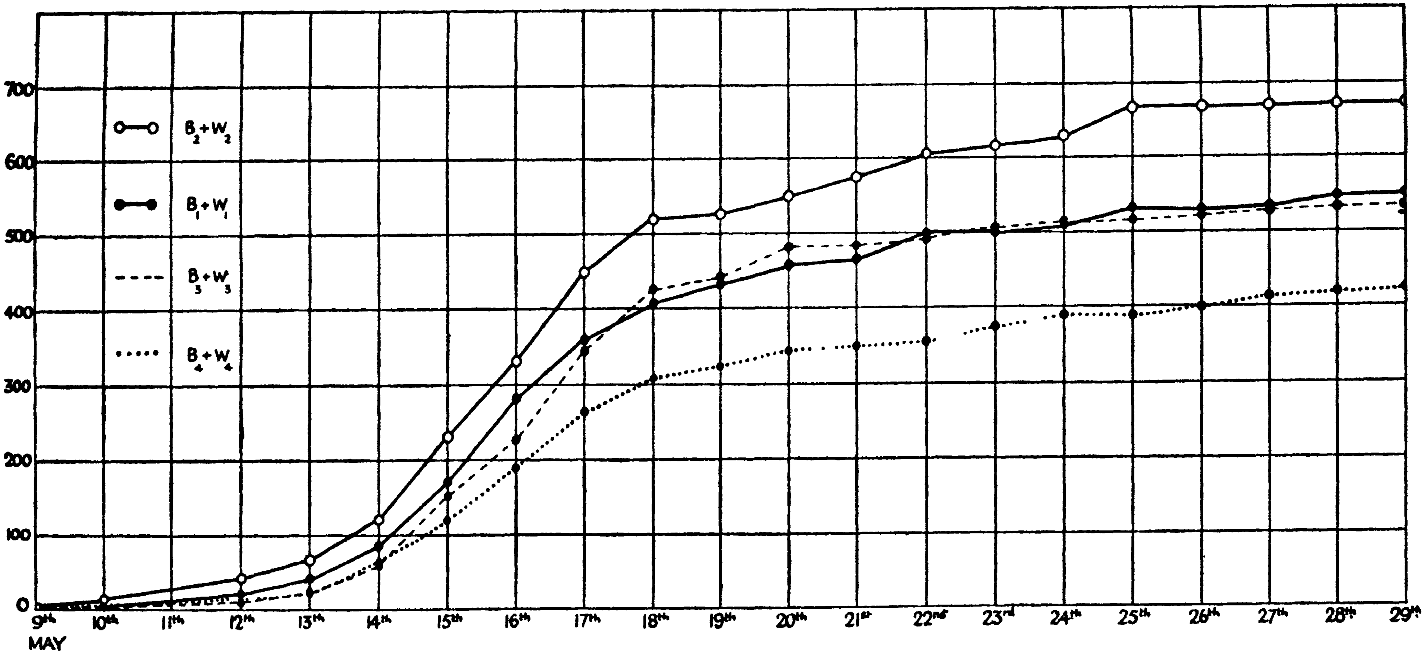

FIG 5.

No of Flowers which have opened by stated dates (positions in plot compared).

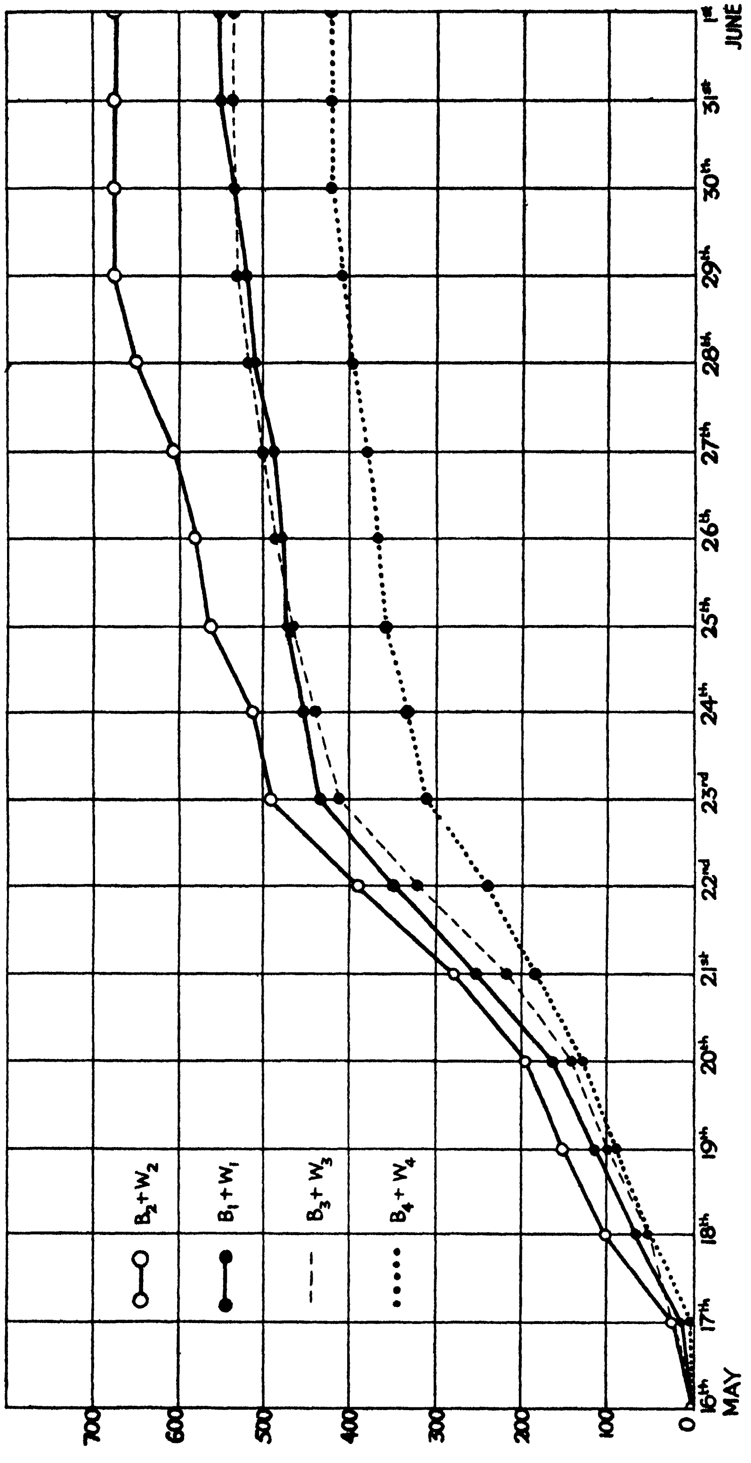

Fig 6

No of Flowers which have dropped (all petals) by stated dates (positions in plot compared)

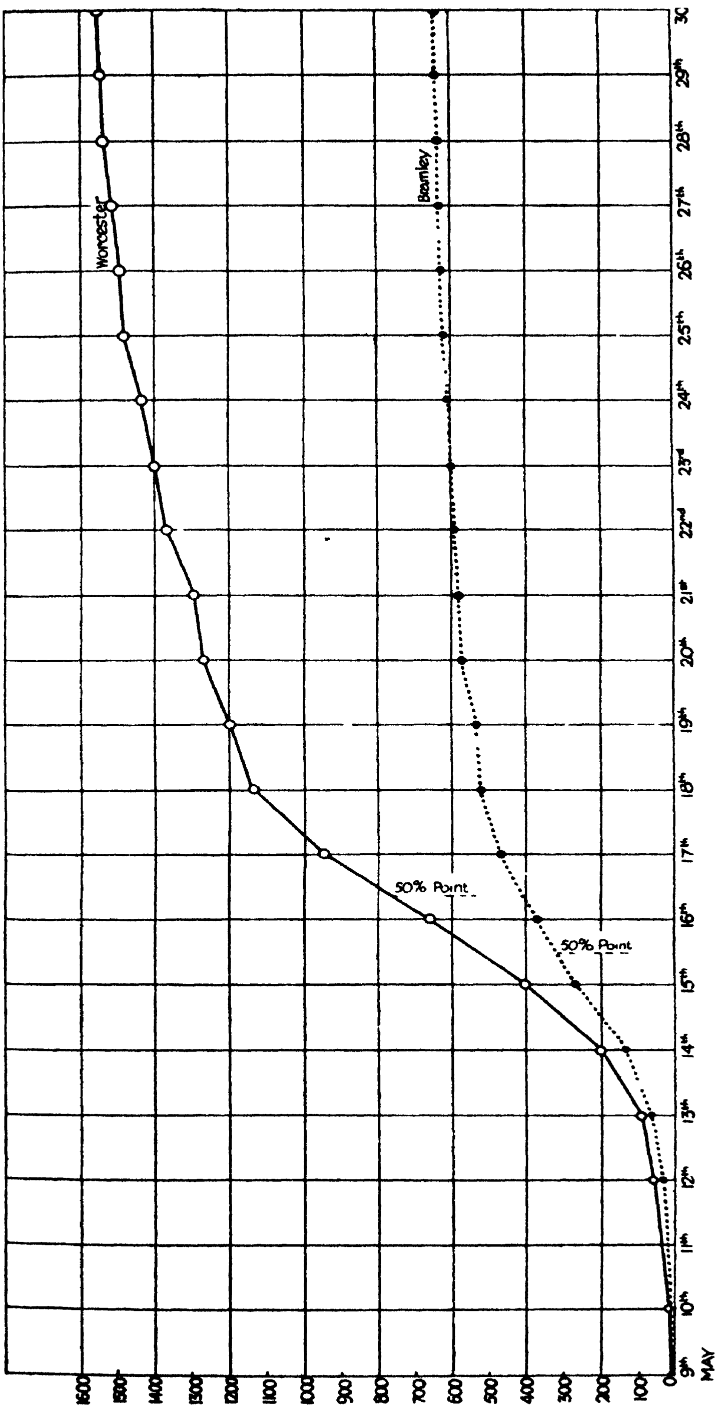

FIG. 7.

No. of Flowers which have opened by stated dates.

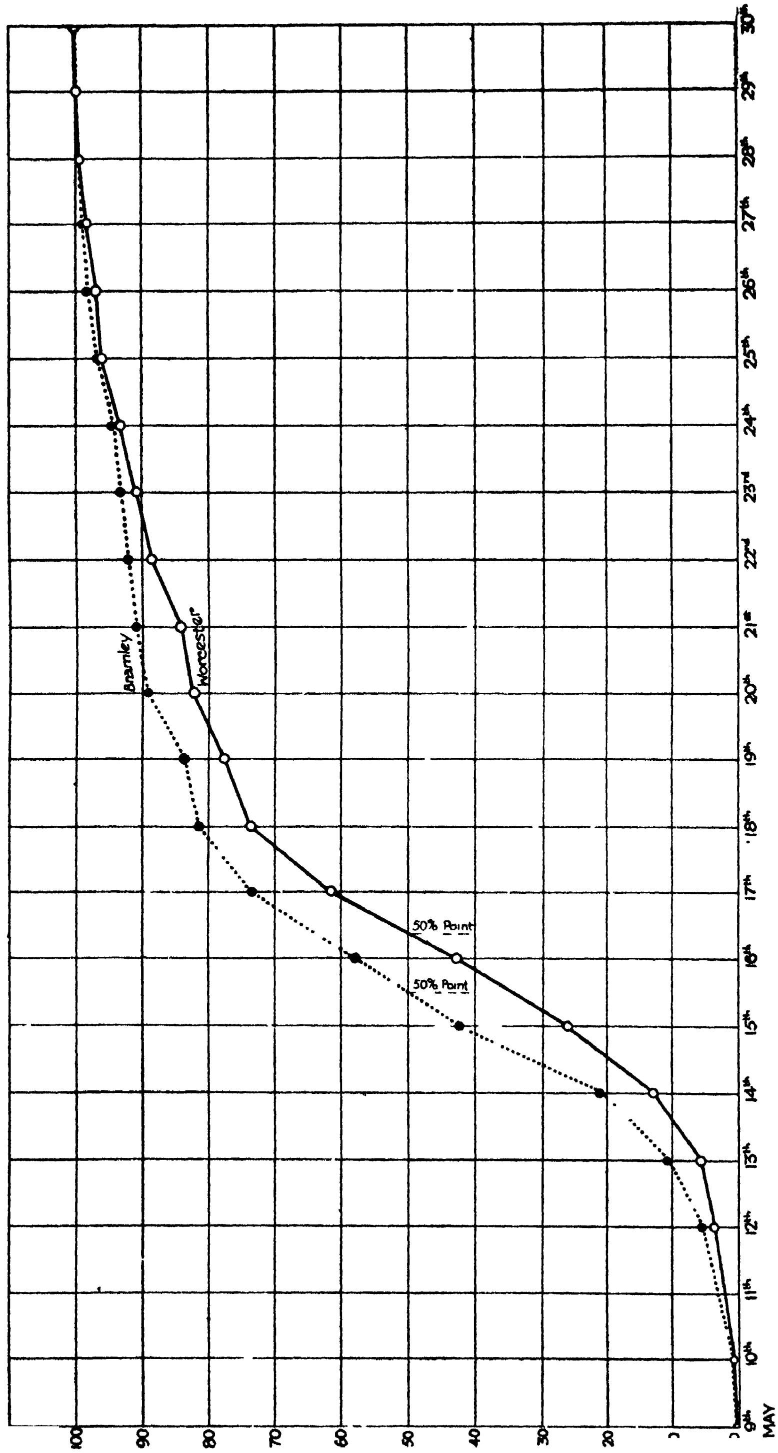

FIG. 8.

Percentage of Flowers which have opened by stated dates.

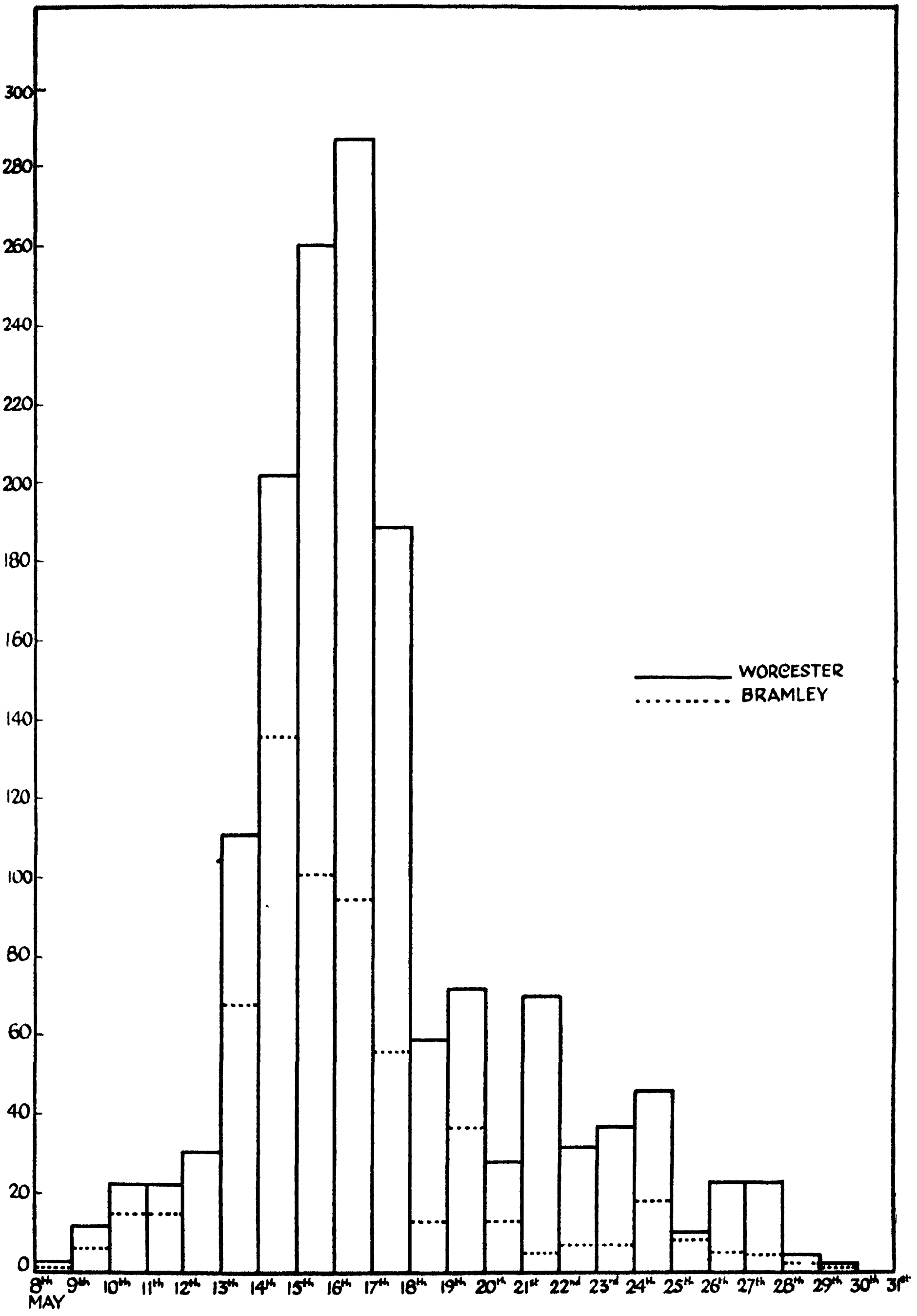

FIG. 9.

Frequency Distribution of Time of Flowering (actual frequencies)
No. of Flowers which opened each day.

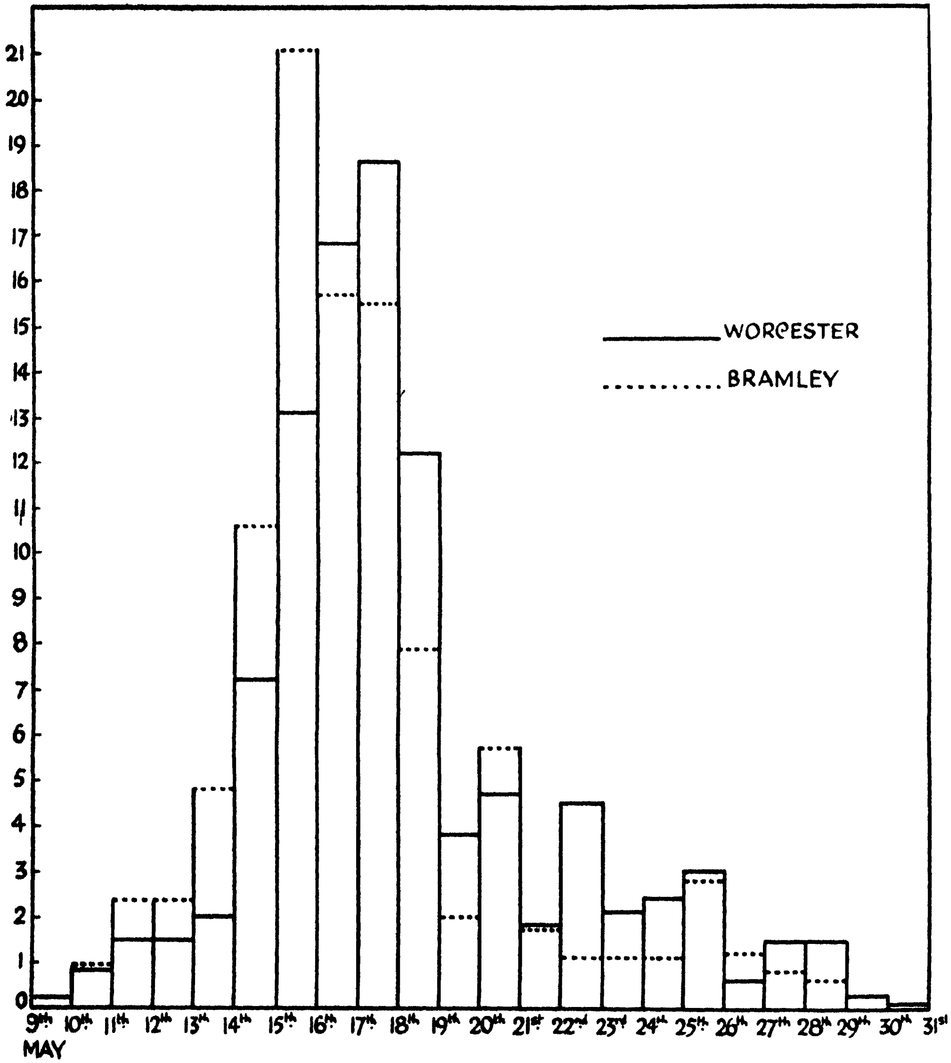

Fig. 10

Frequency Distribution of Time of Flowering (percentage frequencies).
Percentage of Flowers which opened each day.

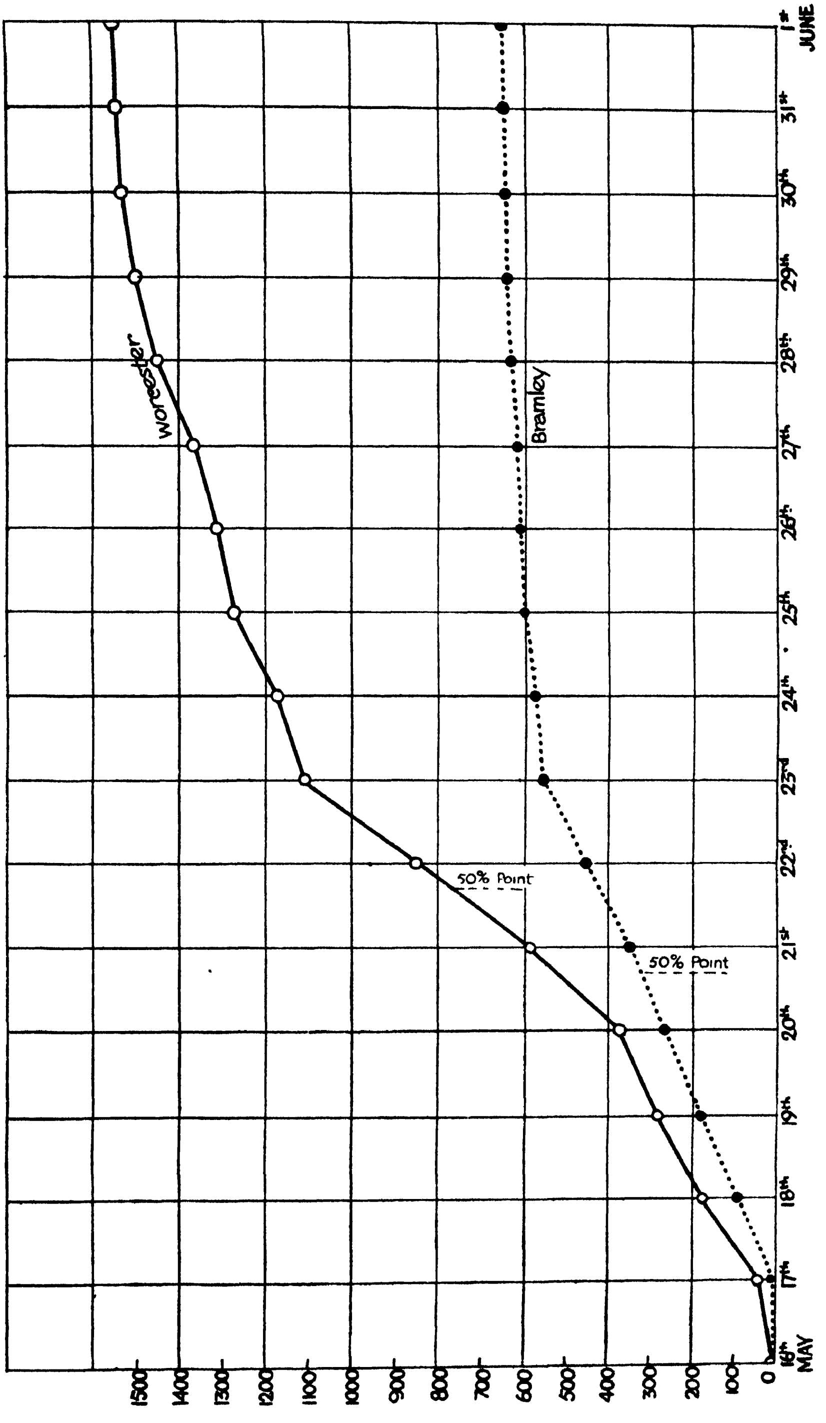

FIG. 11.

No of Flowers which have dropped (all petals) by stated dates

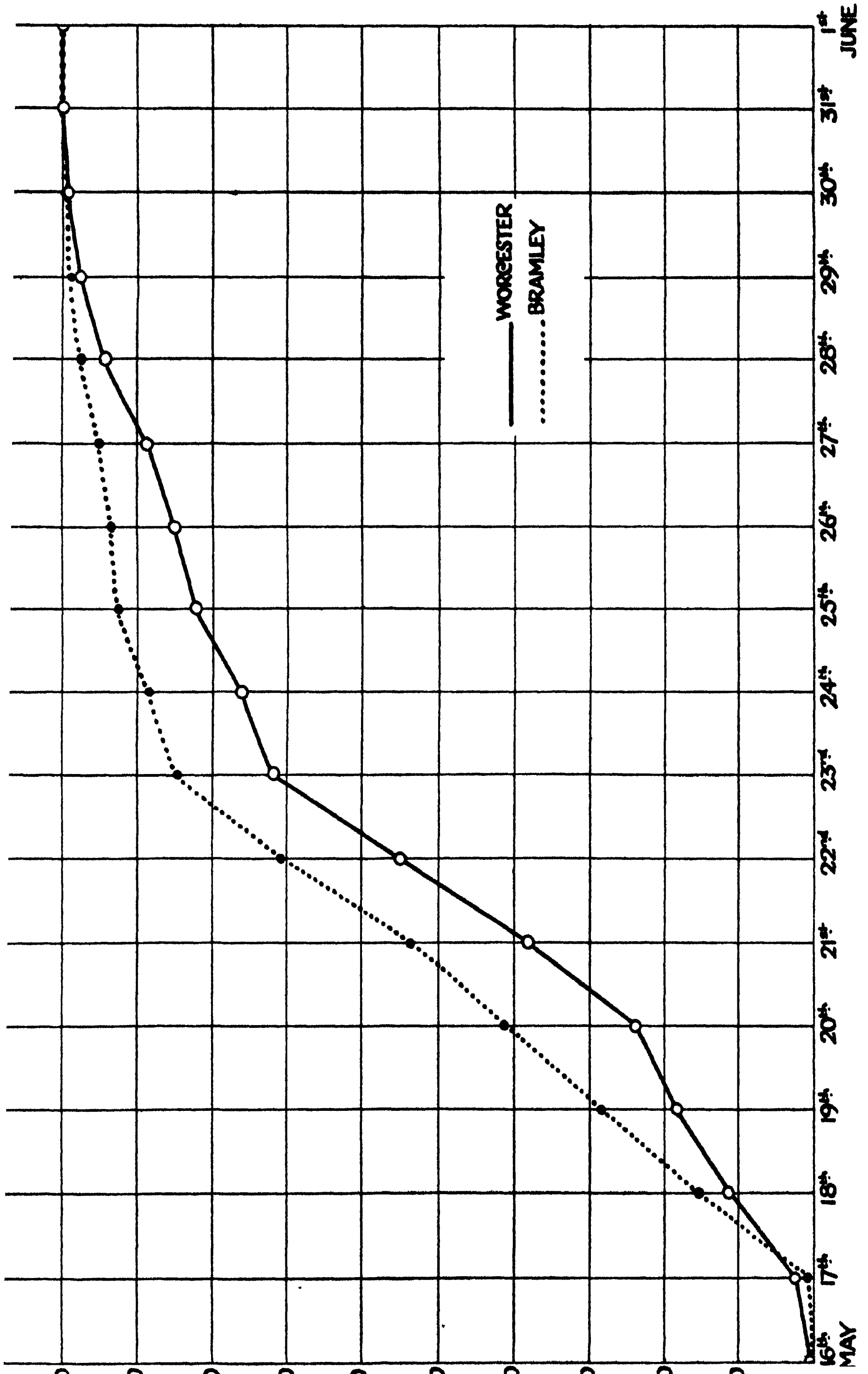

FIG. 12.

Percentage of Flowers which have dropped (all petals) by stated dates.

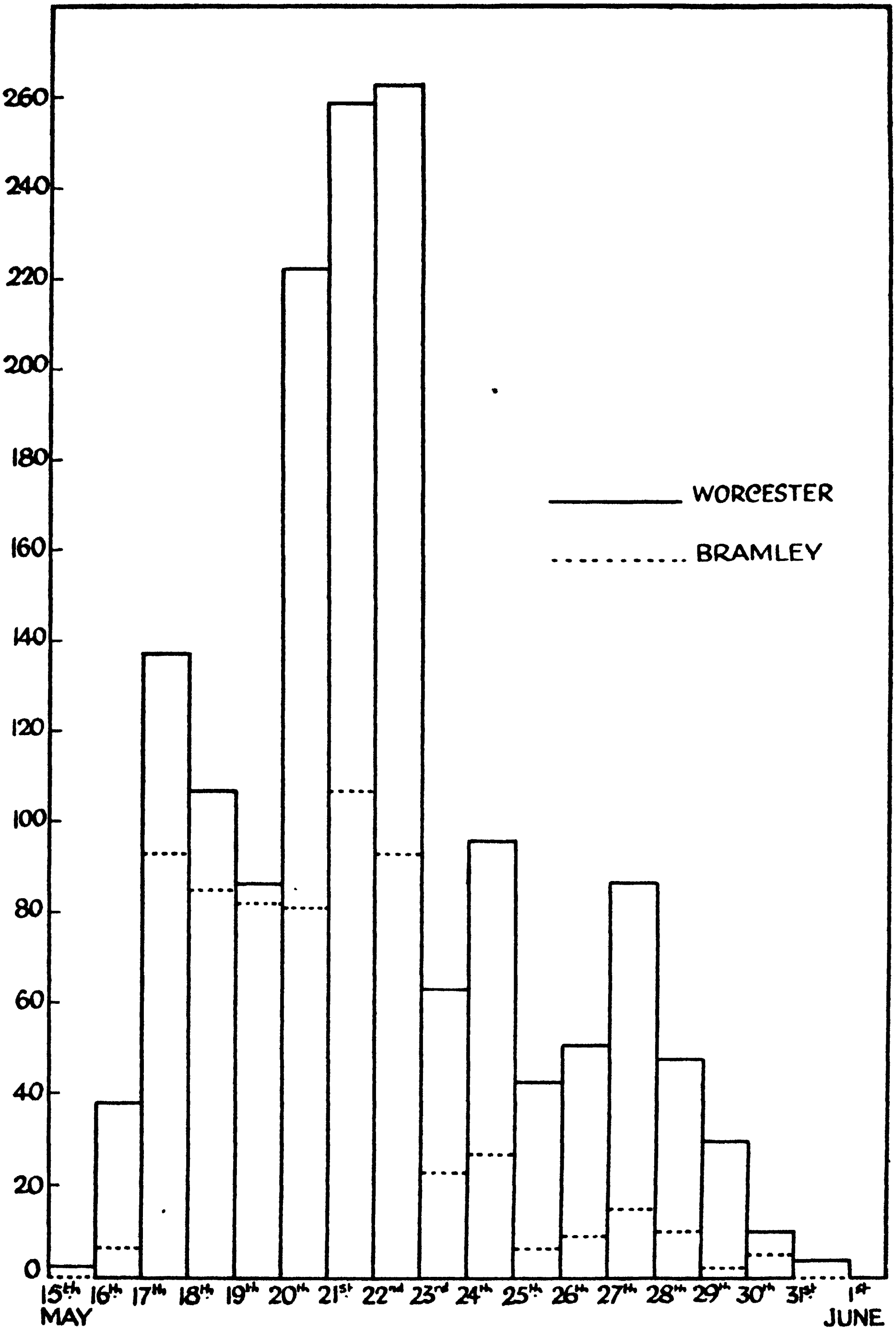

FIG. 13.

Frequency Distribution of Time of Drop (actual frequencies).
No. of Flowers dropping (all petals) each day.

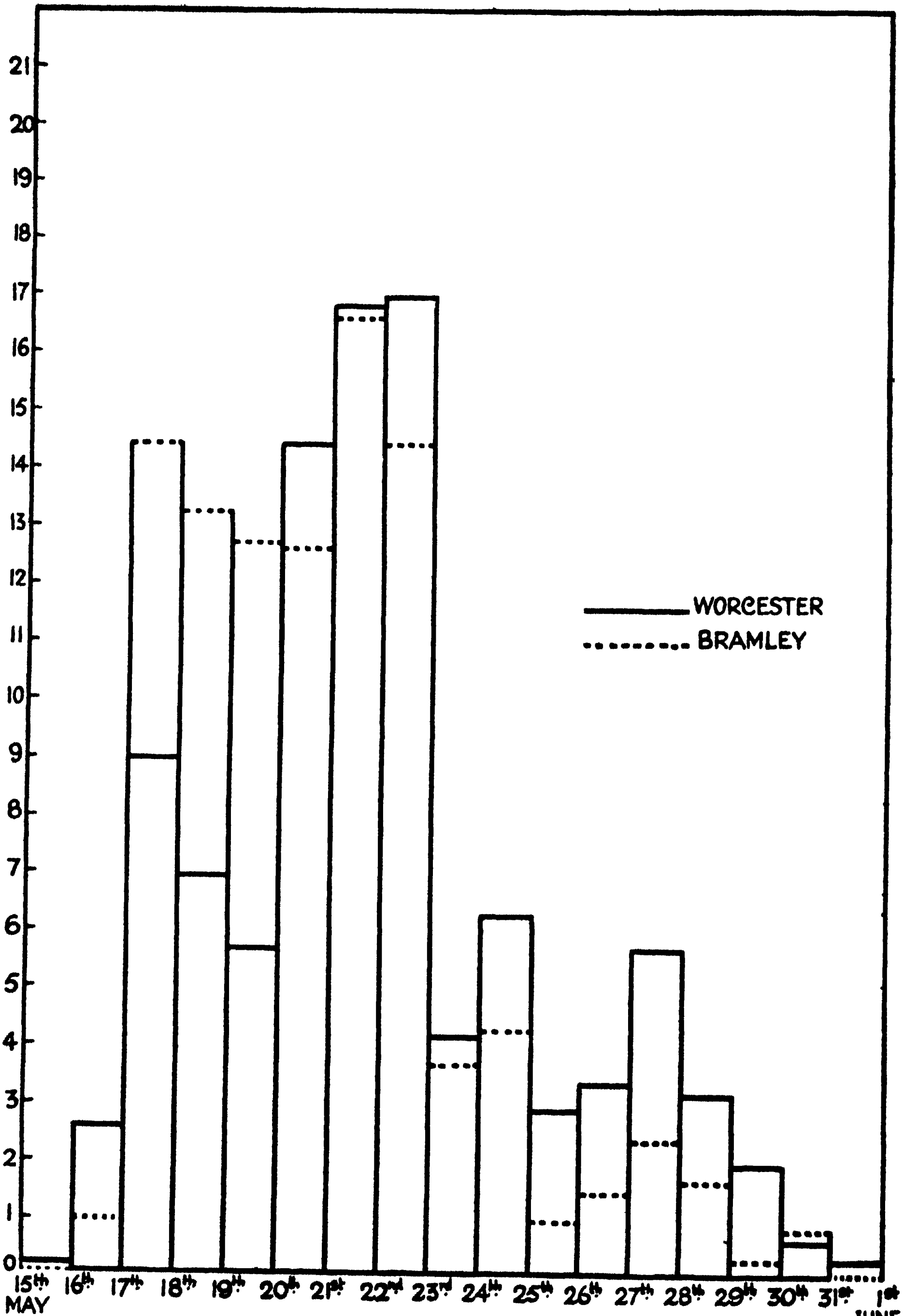

Fig. 14.

Frequency Distribution of Drop (percentage frequencies).
Percentage of Flowers dropping (all petals) each day.

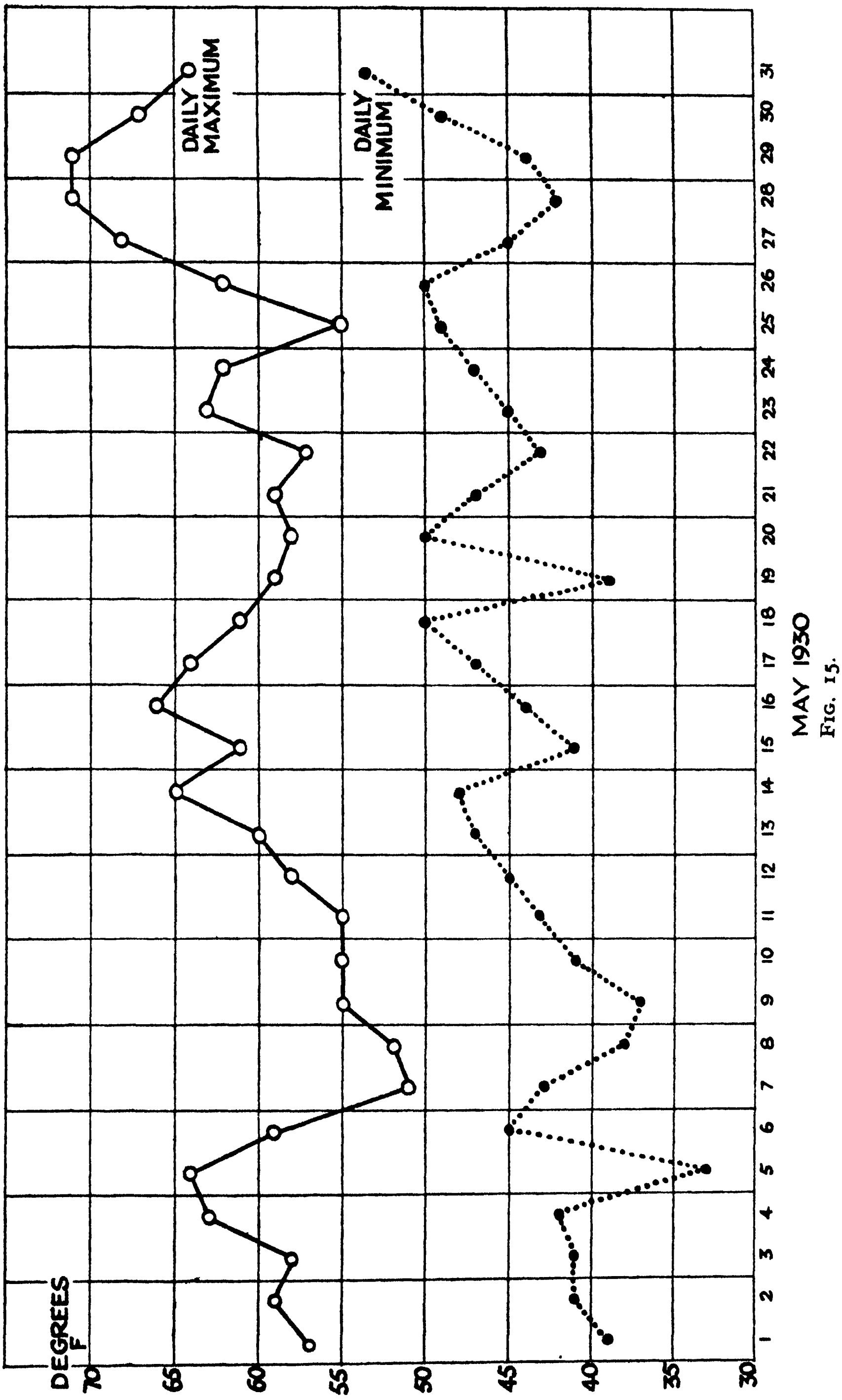

FIG. 15.

Daily Maximum and Minimum Temperatures—May 1930.

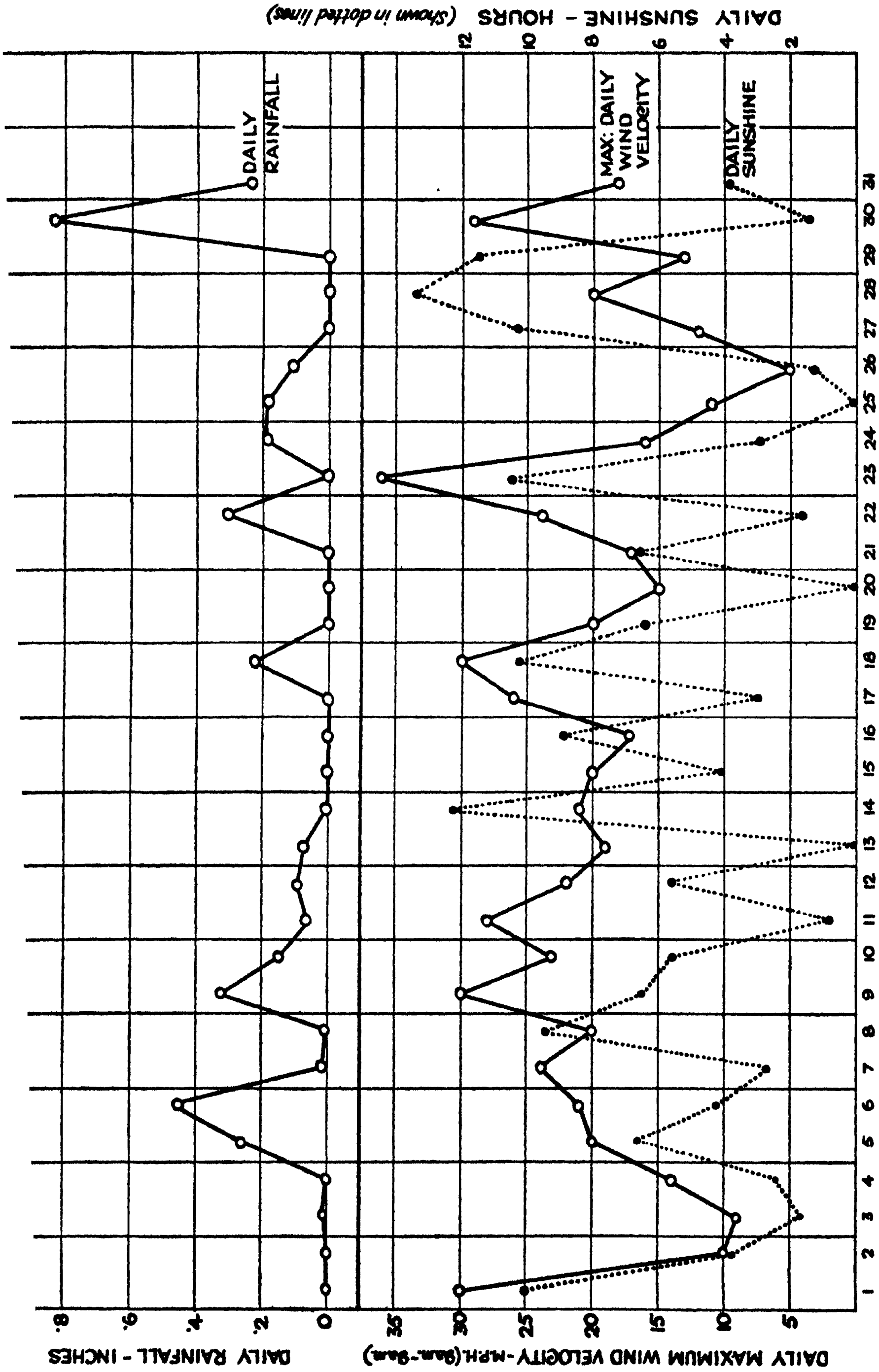

Fig. 16.
Rainfall, Wind and Sunshine—May, 1930.

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INFLUENCE OF SIZE AND SHAPE OF PLOTS ON THE PRECISION OF FIELD EXPERIMENTS WITH POTATOES.

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BIBLIOGRAPHICAL REVIEW.

A SHORT summary of the comparatively few investigations of this kind is given below. As the problem is somewhat different from that of cereals, I have confined myself to the papers dealing with potatoes.

Mitscherlich (5) showed that the probable error per cent. diminishes when the area of the plots is increased. In his experiments the plot sizes varied from 3·6 sq. m. (38·8 sq. ft.) to 237·6 sq. m. (2557·5 sq. ft.), the probable error varied from 5·0 to 1·7 per cent.

When using 4 systematically distributed plots of 25 sq. m. (269 sq. ft.) he obtained, as an average of three experiments, a probable error of 2·39 per cent., which he considers satisfactory. The probable errors were obtained after correcting for soil differences.

Wilson and Chittenden (11) demonstrated the effect of competition. The mean yields per plant increased little less than proportionally to the area occupied per plant. With the variety "Arran Chief" the most remunerative spacing is an interval of 2 to 2½ ft. between rows, and about 15 in. between plants in the rows.

Brown (1) when testing a number of varieties in single-row plots could not detect any influence of competition. He calculated the correlation between the yield of the check rows and the mean of the yields of the two adjacent rows. If competition had any influence, this correlation would have been negative. Actually a small positive coefficient was found namely: 0·271 ± 0·053. Evidently the soil differences concealed any competition that may have been present.

Myers and Perry (8) investigated the difference in yields of hills planted from basal and apical parts of the same tuber. The apical parts were superior to the basal ones, and, owing to competition, the difference was more marked in the rows which were planted closer together.

Moreover they determined the coefficients of variability of plots of different size. The authors conclude that fairly accurate results are obtained when using 5 replications of 25 hill row plots.

Musgrave (6, 7) calculated the coefficient of correlation between check-row yields and yields of adjacent rows, in the same way as Brown. In years when yields were high the coefficient was $-0{\cdot}6526 \pm 0{\cdot}1914$, whilst in years when yields were low, no significant correlation was found. Single-border rows seemed to offer sufficient protection against competition.

Stewart (9), using 3-row plots, found that the border rows gave significantly higher yields than the middle rows in a wet season (1922) but not in a dry season (1923).

In the same way the yields of the southern rows were better than those of the middle rows in 1922, but not in 1923. The effect of border rows evidently depends on the season.

Westover (10), harvested a field, planted with four strains of potatoes, small plots of single rows 10 ft. long. By combining these unit plots the influence of size of plot could be investigated. Only single-row plots were considered.

Although the error continued to drop slightly, the author concluded that only little is gained by using plots longer than 40 ft. In the same way he concluded that the gain in precision by using more than 5 plots was not equal to the additional costs of cultivation.

Kirk and Goulden (3), and Kirk (4), hold that 7 replicated plots should be used. Kirk showed that increased replication was about twice as efficient as increased size of plots. Whilst the variability was reduced 50 per cent. when 4 plots were used instead of 1, the variability was only reduced by 25 per cent. on the average when the lengths of plots were increased from 44 to 132 ft.

Kirk also compared the results of systematic arrangements with a Latin square. When 132 ft. rows and 4 replicated plots were used, the Latin square arrangement showed a probable error 27 per cent. lower than that of the systematic arrangement.

MATERIAL AND METHOD.

The material used for the present study are the yields from a uniformity trial at Ormskirk in 1924.

The field was 201 ft. 6 in. long by 223 ft. 2 in. wide or approximately 1 acre in area. The whole area was planted uniformly with the variety Ninetyfold in 103 rows, the space between rows being 26 in. The field was harvested in single-row plots 33 ft. 7 in. long, 6 of these making up the length of the field, so that there were in total 618 plots. Only

100 rows were used, in order to facilitate the combining of rows into plots of given size, using the same total in each case. The harvesting was completed in 9 days, from the 31st of July till the 8th of August.

In analysing the data a system of randomised blocks was designed; each block consisted of 5 plots, comparable to five treatments or varieties. The total variance was then calculated according to Fisher's method of Analysis of Variance (2), and the variance due to soil variation between blocks was eliminated. The variation due to "error" and the standard deviation of the single observation were then estimated from the mean square deviation within blocks. By combining the yields of respectively 1, 2, 3, 4, 5, 6, 10 and 20 parallel rows of unit lengths, plots of various sizes and shapes were obtained; each of these again could be combined into plots of different lengths, namely, 1, 2, 3 and 6 times 33 ft. 7 in., so that the total 32 different arrangements were considered.

In each case Fisher's "*z*" test was used to see whether the variation was diminished by taking out variation due to blocks.

To see what would be the effect of discarding border rows the same calculations were made for all plots bigger than 2 rows, using only the yields of the inner rows. Obviously the same total area was not used for different plot sizes, as when 3-row plots were used only one-third of the area was actually considered, with 4-row plots half of the total area was used, etc. This point will be seen to be of some importance.

It will be noted that in all cases where 3 or 6-row plots were used, only the first 90 rows could be taken into consideration instead of the total 100. Consequently the number of replications is slightly smaller than it would have been if the same total area could have been used for all combinations.

EXPERIMENTAL RESULTS.

The influence of size and shape of plots on the precision of the experiment can be studied from the following table of standard deviations expressed as a percentage of the mean yield.

It will be noted in Table I that the standard deviation first decreases with increasing width, until the plot is 4 rows wide, and from that point rises when the plots are widened still more.

The initial decrease with increased size of plots confirms the results of all former experiments of this kind, but the subsequent rise is not commonly observed.

It is very probably a result of the method used in analysing the data. When small plots are used a much bigger part of the soil variation can be removed than when bigger plots are used. When plots are increased there are thus two opposite tendencies: a decrease of error as a result of averaging out soil differences and an increase of error due to the fact that more variation is included within blocks. The former tendency being the most important in small, and the latter in big plots, a minimum naturally results.

Table I. *Standard deviation of a single plot as a percentage of the mean.*

Width of plot in rows, 26 in. wide			Length of plot in units of 32 ft. 7 in.			
			1	2	3	6
	Harvesting entire area	1	10·08	8·21	7·32	6·36
		2	8·17	6·93	6·39	5·76
		3	9·02	8·03	7·57	7·06
		4	7·31	6·38	5·75	4·91
		5	7·92	7·01	6·45	5·83
		6	7·92	7·82	6·86	6·33
		10	9·44	8·63	8·20	7·80
		20	8·64	8·08	7·69	7·37
	Harvesting central rows	3	10·84	9·22	8·40	7·62
		4	8·91	7·78	7·01	5·94
		5	9·23	7·72	6·86	6·08
		6	8·73	7·93	7·57	6·81
		10	10·57	9·64	9·20	8·67
		20	9·37	8·83	8·50	8·11

In fact, when the plots are 10 and 20 rows wide, the difference in variance, as estimated before and after the variation between blocks has been removed, is insignificant.

Another point of interest is, that when, instead of 1-row plots, 2-row plots are used, the standard deviation drops considerably, but when 3-row plots are used the decrease in the standard deviation is much less. It seems that this indicates the influence of competition between rows; a high-yielding row will probably depress the yields of the adjacent rows, in this way giving a high variation between rows. When these rows are paired the pairs will tend to give an average with less variation than the single row.

When 3 rows are combined, however, the total yields need not tend to give a uniform average.

The third conclusion that can be drawn from the data is, that a much lower standard error is obtained when the length of the plots are increased than when widths are increased correspondingly. This is due to "slicing up" of the local differences when long plots are used, so that the differences are more distributed over different plots than is the case when the plots are wider. In other words: parallel rows are more correlated than rows only touching at the ends, and therefore, when combined into squarish plots, do not tend to average out the differences as much as long and narrow plots.

Finally it will be noted that the plots, when border rows were ignored, showed much higher errors than those when the total area was harvested. As in the former cases, a smaller part of the soil was used than in the latter, this was to be expected.

The standard deviations in Table I are standard deviations of the single observation. The standard deviations of the means will of course show a different aspect, as the number of replications has to be considered. It is possible to calculate the "efficiency" of the arrangement, defining the efficiency as a quantity measuring the total amount of information suggested on a given area.

It has been generally observed that more is gained by increased replication of smaller plots, than by the use of bigger plots with less replications. It is therefore probable that the efficiency will decrease as the size of plots is increased. The amount of information being the same, plots twice as big will require twice as much land, and therefore will be only half as efficient.

The efficiency can thus be found by multiplying the variance per plot by the number of unit plots that were used to make up the plot, and taking the reciprocal.

Taking the efficiency of the unit plot as 100 per cent., the efficiency of the other plots can be calculated, giving the results in Table II.

Table II. *Efficiencies of plots of varying size and shape.*

Length of plot in units of 33 ft. 7 in.	Width of plots in rows, 26 in. wide													
	Harvesting entire area								Harvesting central rows only					
	1	2	3	4	5	6	10	20	3	4	5	6	10	20
1	100 %	76	42	48	32	27	11	7	28	32	24	22	9	6
2	75	53	26	31	21	14	7	4	20	21	17	14	6	3
3	63	29	20	26	16	12	5	3	16	17	15	10	4	2
6	42	26	11	18	10	7	3	2	10	12	9	6	2	1

The great advantage of many small plots above fewer big ones is seen at a glance from this table. When only central rows are harvested the table of efficiency shows the interesting fact that a greater precision is obtained from 4-row plots than from 3-row plots. This is because of the fact before mentioned that only one-third of the total area is actually used for the calculations in the case of 3-row plots, whilst half is used when 4-row plots are chosen. There would then be no advantage in using 3-row plots when border rows are to be ignored, although a greater number of replications would have been available.

It is obvious that the most desirable plot size cannot be determined from this quantity only; the most economic size of plot will depend on several other factors as well, as, for example, the value of land, costs of cultivation, etc.

Finally, the influence of shape of plots can be seen from Table III where the efficiencies of plots of different shape but with the same area are compared.

Table III.

Approximate area of plot (sq. ft.)	Approximate ratio length : width	Efficiency %
145	31 : 1	75
	8 : 1	76
218	47 : 1	63
	5 : 1	42
290	16 : 1	53
	4 : 1	48
435	93 : 1	42
	3 : 1	27
727	6 : 1	21
	1·6 : 1	11

Generally speaking, the efficiency is considerably higher with long and narrow plots than with plots tending to be more square. There is only the one exception of the 145 sq. ft. plots where shape seems to make no difference. It will be remembered that the long narrow plots in this case consist of a single row, whilst the plots of the other shape are 2-row plots. This is a further indication of competition, the gain in precision by taking longer plots is lost by the greater variation between single row plots.

SUMMARY AND CONCLUSIONS.

1. A uniformity trial with potatoes was used for investigating the effect of size and shape of plots on the precision of field experiments. Up to a certain limit the S.D. in per cent. of the mean decreases when the size of plots is increased; further increase of plot size increases the errors as a lesser part of the soil variation can be removed.

2. Two-row plots show less variation than either 1 or 3-row plots. This may be explained by row competition.

3. When the area to be used is fixed, smaller plots are more efficient than larger, owing to the greater number of replications in the former

case. One exception occurs in the case where border rows are not harvested; here 4-row plots are more efficient than 3-row plots, owing to the fact that a larger part of the soil is included in the calculations when 4-row plots are used.

4. Long and narrow plots are more efficient than shorter and wider of the same size. The only exception is again explained by row competition.

5. In field experiments with potatoes fairly large plots should be used; at least 2 rows wide and preferably long and narrow strips.

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EXPERIMENTAL ERROR AND THE FIELD-PLOT TECHNIQUE WITH POTATOES.

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(With One Diagram and One Text-figure.)

Section I.

Introduction.

Agronomists have long recognised the fact that the plots of an experimental field may differ considerably among themselves. This variability is the source of the greatest difficulty in the interpretation of the results. The studies in the field-plot technique carried out by numerous writers have shown that heterogeneity is a practically universal characteristic of experimental fields and that it must be considered in the interpretation of the results of all plot tests.

The realisation of the lack of uniformly productive land for comparative crop tests has given rise to a number of methods frequently used for ascertaining and overcoming the resultant experimental error. The use of frequent systematically distributed check plots planted to a uniform crop for the purpose of (*a*) indicating the degree of variation due to the soil, or (*b*) correcting the results from the intervening test plots, has often been used as one of the chief methods. The importance of replication and of the use of the best size and shape of plot is also recognised in reducing the experimental error. The earlier work suffers, however, from the defect that systematic replication was resorted to which does not give a valid estimate of error. Dr Fisher has shown the dangers of systematic replication, and advocates the use of random distri-

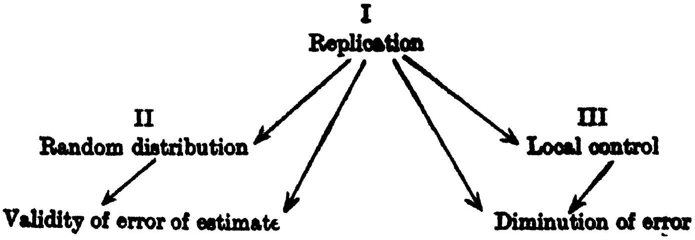

Diagram 1.

bution. Dr Fisher's diagram in the Statistical Laboratory at Rothamsted indicates clearly the principles of modern field experimentation (Diag. 1).

The aims of the experimenter are to get (i) the valid estimate of error and (ii) an error as small as possible. Replication is at the basis of both these aims. The random distribution of plots is concerned only with getting a valid estimate of error and "Local Control"—the field technique with its actual reduction together with all other precautions to obtain accurate results.

Material and method.

The material for this study has been kindly supplied by Dr L. E. Kirk, Professor of Field Husbandry, University of Saskatchewan. He has already published an interesting discussion of this material (1).

In his study consideration has been given to the value of replication in reducing experimental error, size of plot in relation to the number of replications, and to the variability of yields when the plots in each replication series follow the usual systematic distribution, as compared with the result when they are arranged at random in the Latin square.

The object of the present investigation is to study exhaustively by modern statistical methods the effect of size, shape and arrangement of plots on the experimental error with potatoes.

The data consists of 96 rows, each 132 ft. long, planted to a uniform strain of Early Ohio potatoes one seed piece to a hill, the latter being spaced 2 ft. apart in the row and 3 ft. between the rows. Each row was harvested in six units, each unit being 22 ft. long. There are, therefore, 576 one-row plots one unit long. The yields of these ultimate plots are given in the appendix.

The statistical technique, known as the "Analysis of Variance" (2), devised by R. A. Fisher, has been used for the present study. The principle of the method is that the total variation between the individual results in a set of data, as measured by the sum of squares of deviations of these results from their general mean, is made up of the sums of squares of deviations for the various causal factors and a part due to unknow uor uncontrolled causes. It is this latter fraction which provides a logical basis for an estimate of the errors of an experiment.

Analysis of the data.

The analysis of variance of yield is made on the assumption of six varieties or treatments to be tested in order that the major portion of soil heterogeneity may be eliminated. The yields of ultimate plots are combined in different ways and the experimental error is determined from the variation between plots within blocks. The yields of all plots are used except when 3 and 5-row plots of different lengths are studied, in which case the first 90 consecutive rows only are considered. Blocks were formed by grouping adjacent row plots.

The sum of squares due to the variation between blocks can be removed from the total sum of squares, representing as it does the mean fertility differences between the blocks. The balance represents the sum of squares of deviations due to the variation between plots within blocks and can be used as the basis for the calculation of the estimate of error of the experiment. Usually an appreciable portion of the total variance can be removed by this method of randomised blocks. Working on the basis of variation between plots within blocks, one ordinarily gets a lower experimental error which greatly increases the precision of the experiment.

The result of the analysis of variance for 1-row plots 1 unit (22 ft.) long are given in Table I. As observed above, six varieties are assumed for the study. There being 576 ultimate plots, we have 96 replication series or blocks which give 95 degrees of freedom for the variation between blocks. There are 5 degrees of freedom for the variation between plots within each block, giving (5 × 96) 480 degrees of freedom for the variation between plots within blocks making a total of 575 degrees of freedom for the 576 yield figures. As the experiment is planted to a single variety, the variation between varieties with 5 degrees of freedom has no meaning and is not taken out.

Table I. *Analysis of variance of weight of potatoes in single-row plots* 1 *unit long.*

Due to	D.F.	Sum of squares	Mean square	$\frac{1}{2}\log_e$
Between blocks (6 row × 1 unit)	95	6950·9031	73·1674	2·14631
Within blocks	480	1993·270	4·1526	0·71187
Total	575	8944·1731	15·5551	—

$z = 1{\cdot}43444.$

The total sum of squares was obtained by summing the squares of all the 576 plot yields and substracting from it the product of the general mean and the general total. The sum of squares between blocks was obtained by summing the squares of the total weight of each of the 96 blocks, dividing it by 6—the number of elements contributing to each total—and subtracting the same product of general total times the general mean. The difference between the two represents the variation between plots within blocks.

The mean square or the estimate of variance (square of the standard deviation) is obtained by dividing the sum of squares by the appropriate number of degrees of freedom. The standard deviation is the square root of the mean square.

It will be observed from Table I that the blocks have removed a considerable portion out of the total sum of squares. Fisher's "*z*" test is used to test this significance. "*z*" is half the difference between the natural logarithms of the two variances and its sampling error depends only on the number of degrees of freedom on which the variances are based. Tables (3) have been provided for two different levels of significance, the 5 and the 1 per cent. points. If the 5 per cent. point of "*z*" is reached, it is to be understood that as great a difference between the two variances as was actually observed, would only occur by chance from homogeneous material, once in 20 times. For the calculation of "*z*" for the values not included in the table, Dr Fisher's formulae were used.

$$\text{"}z\text{"} = \frac{0{\cdot}6449}{\sqrt{n-1}} - 0{\cdot}7843\left(\frac{1}{n_1} - \frac{1}{n_2}\right) \text{ for the 5 per cent.,}$$

and

$$\text{"}z\text{"} = \frac{2{\cdot}3263}{\sqrt{n-1}} - 1{\cdot}235\left(\frac{1}{n_1} - \frac{1}{n_2}\right) \text{ for the 1 per cent. point,}$$

when n is the harmonic mean of n_1 and n_2, n_1 is the number of degrees of freedom corresponding to the larger variance, and n_2 the degrees of freedom for the other.

A similar analysis was carried out for plots of various shapes and sizes formed by combining the yields of respectively 1, 2, 3, 4, 5, 8 and 16 parallel rows of unit lengths. Each of these again were combined into plots of different lengths namely, 1, 2, 3 and 6 times 22 ft., so that the 28 different arrangements are considered.

Table II. (*Harvesting entire area.*) *Standard deviation of a single plot in per cent. of the mean.*

Width of plot in rows 3 ft. apart	Length of plot in units of 22 ft. each			
	1	2	3	6
1	8·76	6·85	5·97	4·39
2	7·17	6·63	6·17	5·36
3	8·51	7·72	7·22	6·76
4	7·15	6·18	5·54	5·11
5	8·09	7·42	6·42	6·14
8	12·96	12·34	11·77	11·59
16	14·72	14·42	14·07	13·67

Result of the analysis. The influence of the size and shape of plots on the precision of the experiment can be studied from Table II.

Size of plot.

It will be observed from Table II that within the same general shape there is a slight reduction in error due to the increase in the size of the plot up to plots 4 rows wide, but any further increase in the width of the plot results in the increased standard error. The initial drop in the standard error with the increased size of plots is quite in agreement with the results of previous experiments on field plot technique, but the subsequent rise is not ordinarily observed.

From Table I it is evident that the blocks (6 rows × 1 unit wide) have largely contributed to the total sum of squares. The design of the experiment in this case has resulted in the diminution of the variance by 73·3 per cent. This phenomenal reduction, however, is not brought out when plots more than 4 rows wide are grouped into blocks of correspondingly larger size. In fact, when plots 16 rows wide were used, the blocks did not remove any appreciable portion of the total variance. The estimate of standard error after allowing for the variation between blocks is more than that obtained from the total variance.

The result of 16 rows × 1 unit plot is given below.

Table III. (1 *row* × 1 *unit plot basis.*)

Due to	D.F.	Sum of squares	Mean square
Between blocks (96 × 1)	5	196·0908	39·21816
Within blocks	30	5633·8835	187·79612
Total	35	5829·9743	166·57069

Thus, two opposite factors are at work when the size of the plot is increased: first a tendency to average out soil differences with the consequent reduction in the error, and secondly, the increase in the soil variation within blocks, the former predominating in small and the latter in the larger plots. In this connection it would be of interest to study the contour map of this field.

The fertility contour map of the field is shown in Fig. 1. It provides a graphic illustration of the effect of soil heterogeneity on the yield. The original yield given in the appendix is for convenience, combined to form 6-row plots 22 ft. long. In the construction of these contour maps it is assumed that the average yield of each plot is at its centre, and points at which the yields are 5, 10, 15, etc. per cent. below or above the average are found by interpolation between adjacent plots.

It will be observed that the first one-fourth of the section of the field has running through it the contour lines of 5, 10, 15, 20, 25 and 35 per cent. above and the other three-fourths of the field has contour lines 5, 10 and 15 per cent. below the average. Another interesting feature of the first one-fourth section of the field is that the contour lines are quite in the descending order and the zero contour lines pass through the plot 5. It points to the fact that the soil heterogeneity is systematic to a considerable extent.

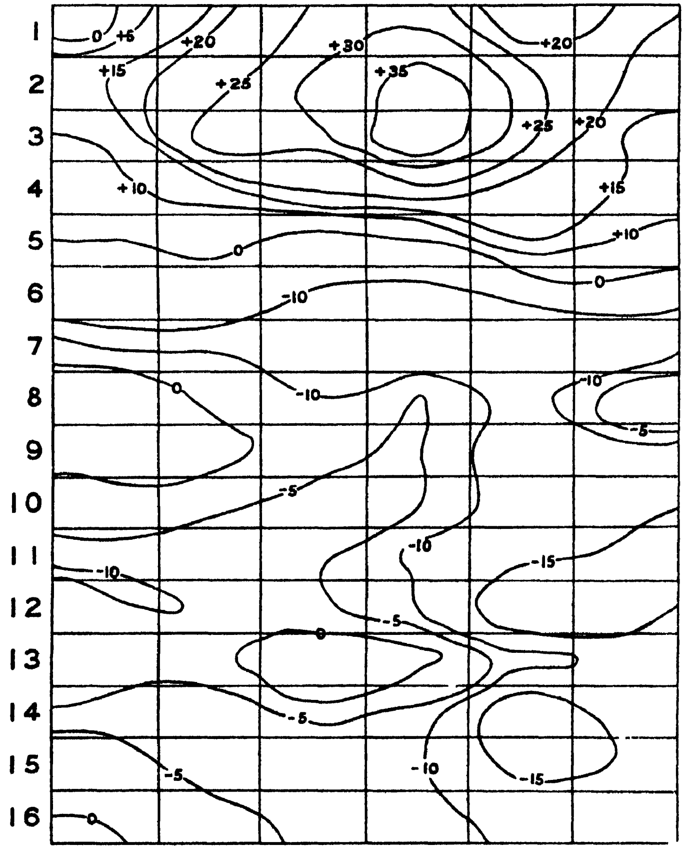

Fig. 1. Contour map of 6-row plots 22 ft. long. Kirk's potato data.

The contour map also clearly shows how the increase in the size of the plot more than counterbalances the averaging tendency and introduces more variation within the blocks when the plots are increased beyond a certain limit.

Referring to Table II again, it would be observed that the 2-row plots 1 unit long have a smaller error than plots 1 row × 1 unit size.

Three-row plots, however, although giving a smaller standard error as compared with the 1-row plot, have an increased standard error in comparison with the 2-row plots.

This is rather difficult of interpretation. The fertility contour lines were such as to give this result. It may be suggestive of the effect of competition between rows. Various agronomists have studied the influence of competition. The literature in some cases is corroborative and in others conflicting. Wilson and Chittenden (4), Myers and Perry (5) and Musgrave (6) demonstrated the effect of competition. Stewart (7) also, when using 3-row plots, found that the border rows gave significantly higher yields than the middle rows in the wet season but not in the dry season, and concluded that the effect of border rows depend on the season. Brown (8), when testing a number of varieties in single-row plots, could not, however, detect any influence of competition.

Shape of the plot.

It will be seen from Table II that the increase in the length of the row is more effective in reducing the variability in yield as compared with the increase in the widths of the plot; in other words, long narrow plots have tended to reduce the effect of soil heterogeneity.

Various workers have paid attention to the study of the effect of the shape of plot in controlling soil heterogeneity with a variety of crops. The conclusions arrived at by different workers are contradictory.

In the classical work of Mercer and Hall (9) at Rothamsted in connection with their Mangold experiment, they came to the conclusion that "little could be deduced as to any superiority of long and narrow plots over square ones." Kiesselbach (10) proved that the variability with oblong plots is smaller than with square ones, while Day (11) pointed out that this is true only if the length of the plot lies along the direction of the greater change of soil fertility, otherwise square plots were preferable. Westover (12) made a study of the variability of potato yields for single-row plots of different lengths from 10 to 150 ft. The error decreased slightly with the increase in the lengths of the row longer than 40 ft.

The review of literature concerning this subject naturally raises the question whether the reduction or increase of the error by the use of long narrow plots is purely a local effect and whether the presence of a pronounced fertility gradient may not accentuate the effect. The reduction of the error may be an indication of the nature of the heterogeneity of the particular field. In cases where the fertility trend is known, the error could then be reduced by elongating plots along the fertility gradient and the blocks of plots across the gradient.

Christidis (13) has studied this problem in detail and concludes that from theoretical considerations the shape of the plots constitutes an important means of controlling soil heterogeneity. He advocates the use of long narrow plots compatible with the practical considerations.

Results of the experiment with the border rows discarded.

A similar analysis to that detailed above was carried out over the whole area, using the yields only of the inner rows to see the effect of discarding the border rows. The standard errors obtained for the various sizes and shapes are given in Table IV.

Table IV. *Standard deviation of a single plot in per cent. of the mean (harvesting central rows).*

Width of plot in rows 3 ft. apart	Length of plot in units of 22 ft. each: 1	2	3	6
3	11·03	9·22	8·40	7·39
4	8·25	7 26	6·68	5·85
5	8·64	7·93	6·88	6·19
8	13·34	12·77	11·97	11·75
16	15·16	14·85	14·44	14·01

The same conclusions as obtained from the study of the plots where entire rows are harvested are brought out when only the central rows are considered. However, the comparison of Tables II and IV show that the standard errors for plots where the border rows are discarded are higher than for those where the yield of the entire rows are taken. This, of course, is due to the fact that the same total area is not used for different plot sizes when the border rows are discarded. For example, only one-third of the area is utilised when the two border rows are discarded in the case of 3-row plots; half when 4-row plots are used and three-fifths in the case of 5-row plots.

Another point of interest emerging out of this study is the comparatively more rapid diminution of error with the increase in the width of the plot up to a certain point than is noticeable when the entire plots are harvested; while increase in the length of the plot with border rows discarded has resulted in the reduction of the error, practically to the same extent as in the case of plots whose entire yields are considered. This point will be referred to later.

SECTION II.

Consideration of the results in Section I clearly bring out the effect of size and shape of plots on the experimental error. It is very often difficult for the agronomist to design a really suitable experiment. Very often the uniform land provided for experimental purposes is rather limited. The problem is to decide on the size and shape of plot, number of replications, and the arrangement of the plots so as to secure the maximum information. Given a piece of land of a certain size, a large number of smaller plots will always assure better results than a smaller number of larger ones down to a certain limit of number and size. It will be seen that what is required is to investigate these limits of number and size of plots with respect to the magnitude of the error they produce. When this is done the available land can then be used to the best advantage.

The "efficiency" of various plots in their use of the land is studied in this section. By "efficiency" is meant a quantity measuring the total amount of information supplied for a given area. It is calculated on the basis of variance per unit area of land. Plots 2 rows wide require twice as much land as single row plots. Plots 3, 4, 5, 8 and 16 rows wide require a corresponding number of times as much land as will single row plots. The "efficiency" can thus be found by multiplying the variance per plot by the number of ultimate units contributing to the total of that plot and taking the reciprocal. Taking the efficiency of the unit plots as 100, the efficiency of other plots can be calculated. The results are given in Tables V and VI.

Table V. *Efficiency of plots of varying size and shape (entire plot harvested).*

	Length of plot in units of 22 ft. each			
Width of plot in rows 3 ft. apart	1	2	3	6
1	100·0	81·7	71·7	66·3
2	74·7	43·6	33·6	22·3
3	35·3	21·4	16·4	9·3
4	37·5	25·1	20·9	12·2
5	23·4	13·9	12·4	6·8
8	5·7	3·1	2·3	1·2
16	2·2	1·2	0·8	0·5

The results detailed in Table V clearly bring out the advantage of greater replication of smaller plots than a smaller number of larger ones. The efficiency in use of land shows in general the tendency to decrease with the increase in the size of the plot. The efficiency drops more suddenly with the increase of the width of the plots than when the length is increased. The importance of the shape of the plot on the control of soil heterogeneity is well marked; for example, the efficiency of 1-row plot 3 units long (1 : 22) is about twice as much that of 3-row plots 1 unit long (1 : 2·4). Similarly 1-row plots 6 units long (1 : 44) are about twice as efficient as 2-row plots 3 units long (1 : 11) and thrice as much as 3-row plots 2 units long (1 : 4·9).

Table VI. *Efficiency of plots of varying size and shape (central rows harvested).*

	Length of plot in units of 22 ft. each			
Width of plot in rows 3 ft. apart	1	2	3	6
3	21·0	15·0	12·1	7·8
4	28·2	18·2	14·3	9·3
5	20·6	12·2	10·8	6·7
8	5·4	2·9	2·2	1·2
16	2·1	1·1	0·8	0·4

The results of Table VI in which only the central rows are harvested show that the efficiencies are greatly reduced as compared to those of Table V where the whole plots were harvested. Another point of interest which emerges from this study is that the 4-row plots are the most efficient in the use of the land when 2 border rows are discarded.

SUMMARY AND CONCLUSIONS.

The present investigation consists of the statistical analysis of a uniformity trial with potatoes conducted by Dr Kirk. In this study the standard error in per cent. of the mean decreased slightly with the increase in the widths up to plots 5 rows wide, but any further increase in the width of the plot resulted in the higher standard error. The fertility contour map of the field is given in Fig. 1 to show graphically the effect of soil heterogeneity on the yield. The increased size of the plot resulted in the decreased efficiency in the use of the land when the entire plot was harvested; in other words, given a piece of land of certain size, it is advantageous to have a greater replication of smaller plots than a smaller number of larger plots. Four-row plots proved to be the most efficient when the border rows are discarded. The superiority of long and narrow plots over shorter and wider ones is demonstrated.

With the greatest pleasure I acknowledge my indebtedness to Dr Kirk, who supplied me with the data, to Dr Fisher for his valuable suggestions and to Dr J. Wishart for useful advice and criticism.

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APPENDIX

Potato plot yields, 1925.

Row	Units					
	1	2	3	4	5	6
1	26·0	25·8	27·6	23·7	26·0	26·0
2	21·2	25·2	29·3	28·4	26·8	27·1
3	26·1	30·5	28·5	32·2	26·8	27·7
4	21·6	27·2	31·0	31·2	28·5	29·6
5	22·2	29·1	29·4	34·9	29·3	32·0
6	21·3	29·6	30·9	34·0	25·8	27·5
7	24·1	29·7	29·6	30·8	24·7	27·4
8	26·6	30·7	30·3	30·5	27·1	29·9
9	28·7	26·8	33·1	30·7	28·1	24·9
10	28·8	33·9	32·4	33·7	31·5	31·5
11	26·8	26·8	31·6	33·5	35·2	27·5
12	27·7	26·2	26·4	32·6	33·4	24·5
13	20·4	29·6	31·9	32·5	31·7	27·1
14	26·8	28·3	29·4	34·8	33·0	27·0
15	27·7	33·5	32·1	31·5	29·5	22·7
16	23·9	30·6	28·9	34·0	31·6	29·1
17	31·3	29·3	26·4	33·7	24·9	27·1
18	23·7	28·0	31·9	29·4	29·0	25·7
19	23·9	25·6	30·8	32·1	26·4	24·2
20	21·6	26·1	28·7	31·1	27·2	24·6
21	29·4	26·9	24·9	30·2	28·3	28·2
22	25·4	27·4	28·0	24·2	26·1	25·6
23	25·6	25·8	29·2	32·5	27·6	28·6
24	26·4	25·7	27·5	24·7	29·3	28·9
25	24·7	25·7	24·4	24·7	28·3	26·3
26	23·4	25·6	24·7	24·1	27·9	25·7
27	22·4	25·1	17·1	23·4	30·4	28·0
28	20·0	23·4	22·4	23·9	25·3	23·8
29	24·1	22·8	21·7	21·7	26·0	24·7
30	23·5	21·7	21·1	20·1	24·7	24·2
31	21·4	21·4	20·1	21·1	22·5	22·9
32	22·8	19·4	21·4	19·5	24·0	21·1
33	19·5	22·2	19·9	20·9	23·1	24·1
34	21·1	22·7	23·0	20·4	22·5	24·6
35	23·5	24·4	21·6	20·2	18·5	19·1
36	22·6	20·1	18·6	20·6	17·6	19·7
37	18·1	21·9	17·9	20·1	17·6	20·0
38	21·4	22·1	17·3	21·3	16·3	18·0
39	19·0	20·2	19·0	20·2	19·4	20·4
40	18·2	19·7	15·1	17·1	18·3	21·5
41	26·3	22·1	19·8	20·9	22·7	16·1
42	21·7	18·3	16·1	20·2	21·0	18·6
43	25·9	22·6	21·2	21·4	17·7	23·4
44	24·6	20·9	19·0	22·5	20·7	24·0
45	24·4	24·6	20·9	23·6	22·6	21·7
46	27·6	20·2	21·0	21·4	21·4	22·4
47	27·3	24·7	22·1	21·8	21·2	22·8
48	26·2	24·9	21·7	22·1	18·4	22·7
49	27·4	26·1	22·2	22·7	22·2	19·9
50	24·1	22·1	21·2	18·7	20·1	21·0
51	24·4	25·1	22·0	22·1	18·4	22·9
52	24·6	21·1	21·9	26·2	19·5	19·7
53	21·2	26·8	22·3	21·7	20·2	20·9
54	23·9	24·0	21·9	21·7	18·2	18·1
55	22·3	22·2	20·9	20·8	19·7	20·3

APPENDIX (*continued*).

	Units					
Row	1	2	3	4	5	6
56	23·8	23·1	25·4	20·6	20·2	19·4
57	22·9	23·2	21·9	23·4	19·6	20·8
58	22·2	21·3	20·6	21·4	19·1	20·4
59	21·6	19·6	20·8	23·1	20·7	20·1
60	22·9	25·1	23·8	23·9	21·4	19·4
61	24·4	22·1	23·1	20·4	20·3	20·4
62	21·4	20·3	22·1	23·0	21·2	20·2
63	21·2	22·5	22·2	19·9	21·2	20·1
64	19·4	20·5	20·4	19·9	17·4	19·0
65	21·9	21·2	26·2	23·9	19·0	18·4
66	20·6	20·9	20·1	17·3	21·9	19·7
67	17·0	18·0	21·2	22·7	16·6	17·6
68	21·7	20·2	19·7	20·8	17·7	19·4
69	21·6	23·8	22·6	19·6	18·1	17·5
70	18·5	22·5	20·2	18·9	19·8	19·9
71	21·6	22·8	23·9	23·5	20·0	19·1
72	18·5	21·2	26·4	20·6	19·9	19·3
73	25·0	24·9	26·9	27·5	22·0	19·5
74	24·2	25·8	24·8	23·7	19·4	22·3
75	24·8	24·7	23·8	23·6	22·3	17·9
76	20·8	19·4	24·2	21·7	19·6	19·6
77	23·8	24·4	24·5	22·5	22·9	22·0
78	18·5	19·2	21·1	24·5	22·5	22·6
79	19·3	22·5	22·5	21·4	19·2	19·6
80	24·2	18·6	22·0	16·3	17·3	21·1
81	20·2	21·3	23·8	23·4	20·5	22·4
82	20·9	20·5	21·0	25·0	20·1	21·2
83	24·7	22·5	22·9	22·9	17·2	17·3
84	20·2	21·7	22·7	20·9	17·7	20·6
85	22·9	15·8	21·7	19·0	18·5	20·2
86	22·3	20·6	22·1	19·4	18·3	18·1
87	21·2	21·9	19·2	21·6	20·2	20·0
88	22·3	24·6	24·1	24·2	20·8	21·4
89	22·2	21·2	16·3	21·4	19·3	19·0
90	24·7	25·2	22·0	20·7	19·3	20·3
91	27·9	24·9	21·9	19·4	19·8	21·2
92	21·1	22·0	22·2	20·8	23·3	21·1
93	21·8	23·0	21·1	19·9	21·9	18·5
94	25·4	23·5	23·1	23·3	19·2	23·1
95	20·4	21·7	20·1	22·8	20·2	21·4
96	23·0	21·6	21·1	20·7	19·2	19·6

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A STUDY IN SAMPLING TECHNIQUE WITH WHEAT.

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(With One Text-figure.)

INTRODUCTION.

IN conducting field experiments in co-operation with farmers, experimental stations very often encounter difficulties in having test plots properly harvested and threshed. Estimation of the yield of experimental plots by harvesting a number of very small, apparently representative areas, consequently becomes desirable and necessary where facilities are lacking for harvesting or threshing accurately the produce of the entire areas.

Various sampling methods for the estimation of yield of cereals have been tested by different workers. Arny and Garber (1) used the "rod row" method. They harvested nine symmetrically placed rod lengths of drill from tenth-acre plots and compared the yields so estimated with those obtained by harvesting the whole plots. They concluded from their studies that nine rod rows removed from tenth-acre plots gave reliable estimates of the yields of the entire plots. Arny and Steinmetz (2) employed the "square yard" method and found that four to five systematically distributed square yard areas removed from tenth-acre plots gave approximately the same standard error for yields as harvesting the produce of the entire plots. Clapham (3) tried three sampling methods; in each case he took 30 samples, each sample consisting of a metre length of drill. In method (*a*) he took ten random samples from each of the three parts into which the entire plot was subdivided, in method (*b*) six sets, each of which comprised a succession of five contiguous metre lengths of drill located symmetrically within the plot, were cut as samples, and in method (*c*) six metre lengths at equal intervals along the plot were harvested from each of the five drill rows chosen at random. He concluded from his studies that a "random sampling" method will provide an estimate of yield with a standard error of about 5 per cent. when 30 samples of metre lengths are taken from one-fortieth acre plot. His investigations also point out the disadvantage of the systematic method of sampling as against the "random sampling" method. He criticised Arny and Garber's "rod row" method on the ground that a rod row is too coarse a unit. In his investigations he found that even a metre is too long, significant correlations having been obtained between successive half-metre lengths of the same drill. He, therefore, advocates taking dissected half-metres as no significant correlation was found to exist between half-metres separated by one metre.

MATERIAL.

The object of the present paper is to design a suitable sampling technique using Clapham's work as a basis. Plot 18 of the Four Course Rotation Experiment, Great Hoos, of the Rothamsted Experimental Station, was selected for the purpose. The plot is about one-fortieth of an acre in size, the actual dimensions being 40·6 links × 60 links, with a path 3 links wide round it. It was sown with Yeoman II wheat. There are 80 rows in the plot, rows being 6 in. apart. The entire plot was harvested in half-metre units. The length of each row is 40·6 links or 8·17 metres. The produce of each single half-metre unit was cut about an inch above the ground with scissors and put into a perforated paper bag which was tied with a string and numbered serially. There were 16 half-metre units in each row, and the extra portion of 0·17 metre left from each row was harvested and bulked so as to get the yield of the entire plot. Sixteen half-metre units numbered in the serial order from each row were tied in one bundle, and 80 such bundles with the produce of the remaining 0·17 metre of each row which was bulked together were then taken to the sample house and suspended from the roof to dry for four weeks. Each sample was weighed before threshing and the number of ears in each sample was counted. A small scale threshing and winnowing machine was used for the purpose (4). The yield of each sample was recorded. The yield of grain, the number of ears and the weight of straw are therefore available from this data. The yield figures are given in Appendix I.

ANALYSIS OF THE DATA.

The analysis of the yield figures brings out clearly some interesting results. It will be observed from the row totals given in the Appendix that the edge rows have given decidedly higher yields than the others. The mean yield of grain per half-metre for rows 1 and 80 is 33·25 and

34·375 gm. respectively as against 18·45 gm. which is the mean yield for a half-metre produce based on the remaining 78 rows. The significance of the difference between the mean yields of edge rows and of the remaining 78 rows could be tested by the t test(5). The sum of the squares of deviations of the 32 half-metres of the two edge rows from the mean is 6392·375 and the sum of the squares of deviations of the 1248 (78 × 16) half-metres is 88865·1008. The pooled estimate of variance is thus

$$(88865{\cdot}1008 + 6392{\cdot}375) \div (1247 + 31), \textit{ i.e. } 74{\cdot}53636.$$

Using the formula

$$t = \frac{\bar{x} - \bar{x}'}{s} \sqrt{\frac{(n_1 + 1)(n_2 + 1)}{n_1 + n_2 + 2}},$$

where $\bar{x}$ and $\bar{x}'$ are the means of the two samples, $(n_1 + 1)$ and $(n_2 + 1)$ the numbers in the samples, and s the standard deviation estimated by pooling the sum of the squares from the two samples and dividing by the total number of degrees of freedom contributed by them, we have $t = 13{\cdot}15$ with 1278 degrees of freedom. The border effect is thus clearly demonstrated.

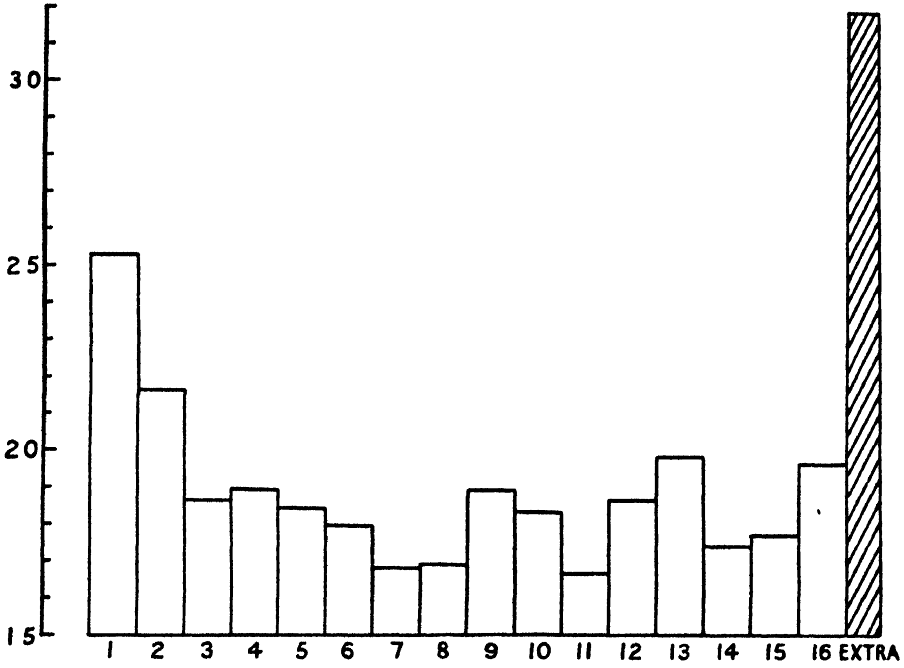
In view of the fact that the border rows have given significantly higher yields, it was thought probable that similar effect would be shown by the end half-metres of each row. The mean yield per half-metre for the 16 sections based on 80 half-metres (there being 80 rows each 16 half-metres long) is plotted in Fig. 1. The mean yield per half-metre as estimated from the extra 0·17 metre lengths for each row is also shown. It is evident that the end portions have yielded higher than the inner ones. There is indication that the alley effect extends to perhaps half a metre within the margin of the plot.

Table I.

Due to...	Degrees of freedom	Sum of squares	Mean square	$\frac{1}{2}\log_e$
Between rows	77	13508·1154	175·4301	1·2823
,, metres	468	28250·9643	60·3653	0·8989
,, half-metres	546	29397·75	53·8420	0·8417
Within rows	1014	57648·7143	56·8528	0·86892
Total	1091	71156·8297	65·2217	—

Having established the fact that there is a significant border effect, further analysis was carried out on figures left after discarding the end rows and end half-metres of each row. There are 78 rows each 14 half-metres long yielding 1092 figures for the study. It is possible to divide the total variation into three parts: (i) Variation due to rows with 77 degrees of freedom, (ii) Variation between metres of the same row with 468, and (iii) Variation between half-metres of the same metre with 546 degrees of freedom. The analysis of variance is given in Table I.

The comparison of the mean squares for variation between rows with 77 degrees of freedom and the mean square for variation within rows with 1014 degrees of freedom clearly indicates that the variation between rows is very much greater than within rows. The value of z is 0·4134, which exceeds even the 1 per cent. point.



Mean yield per half-metre length for the sixteen sections of the row length together with mean yield per half-metre length estimated from the extra length beyond sixteen half-metres

Fig. 1.

The variation between half-metres is smaller than that between metres and indicates that halves of the same metre are to some extent correlated. The difference between the two variances as judged by the z test is not however significant. The value of z is ·0572, while the 5 per cent. value of z is ·0731.

EFFECT OF SUBDIVISION OF THE AREA ON THE SAMPLING ERROR.

It was thought desirable to study how the subdivision of the area reflects on the differences in the mean fertility between the various parts into which the entire area is divided, in order to throw light on the advisability of taking the same number of random samples from each of the parts into which the entire area is divided, with a view to reducing the standard error. The result of the analysis of the variation between and within sub-plots of various sizes and shapes are given below.

Table II. *Dimensions of the sub-plot.*

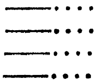
	Between sub-plots		Within sub-plots	
Rows and length in half-metres	Degrees of freedom	Mean square	Degrees of freedom	Mean square
13 × 7	11	334·671	1080	62·477
13 × 14	5	510·649	1086	63·171
26 × 7	5	263·286	1086	64·310
26 × 14	2	421·284	1089	64·568
39 × 7	3	327·511	1088	64·498
39 × 14	1	239·122	1090	65·062
78 × 7	1	0·004	1090	65·222

The comparisons of the mean squares for the variation between and within sub-plots clearly bring out the fact that there is a significant difference between the mean fertility of the sub-plots constituting the entire area harvested, except in the last case where there is no difference between the two sub-plots into which the whole area is divided transversely; when it is divided longitudinally the difference is not quite significant. This indicates that it is advantageous to divide the area to be sampled into a number of parts within each of which an equal number of samples is taken, so as to ensure the proper representativeness of the sample and consequent reduction in the standard error.

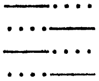
THE SAMPLING TECHNIQUE.

Five sampling methods are studied in the present paper. In each case the ultimate part of a sample designated as a "unit" (6) is a half-metre length of drill. The "sampling unit" comprises four "units" and thus occupies two metre lengths. The five methods, however, differ from each other in the scatter of the four units comprising the sampling unit. The object of using different structures of the sampling unit is to study the variation between and within the sampling units with a view to deciding on a suitable sampling technique. The effect of spacing the units within large sampling units is to make the latter individually more representative of the area sampled, and consequently to achieve a reduction in the magnitude of the error. The five different sampling methods tried are:

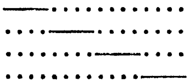
(1) Four parallel half-metre lengths on the adjacent rows:



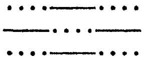
(2) Four half-metre lengths from the four adjacent rows arranged as



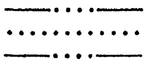
(3) Four half-metre lengths taken end to end in a step-like manner from the adjacent rows:



(4) In this type only three rows are used, but the structure is



(5) Two half-metre lengths separated by half a metre are taken from each of the two alternate rows opposite each other as



It is possible to divide the area into two parts transversely and three longitudinally, giving in all six sub-plots. For convenience, 72 rows, 12 half-metres long, are considered. For methods (1) and (5) four border rows on either side and three half-metre lengths on one side and one on the other are discarded. It must be observed, however, that it is not possible to use the same rows and make the six sub-plots more than approximately identical for all the methods. The shape of the sub-plots, each composed of 36 sampling units compactly fitted together, is different for the different methods. The object is, however, to eliminate variation between the sub-plots, representing as it does the differences in the mean fertility between areas equally sampled, and the sampling error can be determined from the variation between sampling units within sub-plots. Thus determinations will then be made comparably between the different forms of unit tested, in analyses of variance of exactly the same form. The five analyses are as follows:

Analysis of Variance.

		Degrees of freedom	Mean square	$\frac{1}{2}\log_e$
Method (1)				
——····	Between sub-plots	5	252·994	
——····	Between sampling units	210	58·298	0·8815
——····	Within sampling units	648	63·282	0·9225
——····	Total	863	63·168	
Method (2)				
——····	Between sub-plots	5	258·938	
····——	Between sampling units	210	81·098	1·0465
——····	Within sampling units	648	55·712	0·8588
····——	Total	863	63·067	
Method (3)				
——············	Between sub-plots	5	228·771	
····——········	Between sampling units	210	68·816	0·9644
········——····	Within sampling units	648	59·998	0·8959
············——	Total	863	63·122	
Method (4)				
····——···	Between sub-plots	5	163·178	
——···——	Between sampling units	210	74·026	1·0009
····——···	Within sampling units	648	57·073	0·8708
	Total	863	61·813	
Method (5)				
——···——	Between sub-plots	5	245·456	
············	Between sampling units	210	86·264	1·0774
——···——	Within sampling units	648	54·277	0·8458
	Total	863	63·168	

It is interesting to compare the mean squares for the variation between sampling units and the mean squares for the variation within sampling units. In all cases except in method (1) the variation between sampling units is greater than that within sampling units, and as judged by the z test it is significant, except in method (3) where the value of z fails to reach the 5 per cent. point level of significance. It is thus evident that there results a loss of information if the structure of the sampling units is as in methods (2), (4), (5) and perhaps also in method (3), where the half-metre lengths within the sampling units are correlated.

In method (1) the variation between the sampling units is smaller than that within the sampling units. The difference, however, is not significant. The half-metre units within the sampling units are not correlated positively; if anything, there is a negative correlation, which very likely is due to the effect of competition existing between the rows.

From the evidence submitted it seems fair to conclude that method (1), in which the sampling unit consists of four parallel half-metre lengths, is superior to the other methods. It has in this trial the least error, evidently because the units comprising it are not positively correlated. The standard error per sampling unit expressed in percentage is

$$\frac{\sqrt{58\cdot298}}{\sqrt{4}} \times \frac{100}{17\cdot828}, \text{ i.e. } 21\cdot414.$$

To reduce the sampling error per plot of one-fortieth acre to say 5 per cent. it would be necessary to take about 18 such samples. This structure of the sampling unit has another advantage of being simple, and requiring less labour to harvest, as it is easier to cut the four parallel half-metres than when they are separated from each other by measured distances along the rows.

RELATION BETWEEN THE EAR NUMBER AND YIELD PER HALF-METRE.

As already observed, counts were taken on the number of ears per half-metre before the produce of a half-metre drill length was threshed. The original data is given in Appendix II. Only 72 rows 12 half-metres long are considered; the four end rows on either side and three half-metres of each row on one side and one on the other are discarded. A similar analysis to method (1) is carried out on these figures. The area is divided into six sub-plots, into two transversely and three longitudinally. There are thus 24 rows each six half-metres long in each of the sub-plots. The result of the analysis of variance is given in Table III.

Table III. *Analysis of variance on the ear number.*

	Degrees of freedom	Sum of squares	Mean square	$\frac{1}{2}\log_e$
Between sub-plots	5	1056·3530	211·2706	
Between sampling units	210	15217·8125	72·4658	0·99026
Within sampling units	648	38214·7500	58·9734	0·88726
Total	863	54488·9155	63·1390	
			$z = 0\cdot103$	

Table III shows that the variation between the sampling units is greater than the variation within the sampling units. The value z is 0·103 while for the 5 per cent. level of significance the value of z required is only 0·090, showing that the half-metre lengths are correlated as regards the number of ears concerned. This discrepancy may be regarded as supporting the view that the use of four parallel half-metres as a sampling unit is especially accurate for yield by reason of competition between the growing plants, for there is less reason to suppose that such competition should affect ear number.

To study the relationship between the yield and the ear number, covariances were analysed in precisely the same manner as the variances. By covariance is meant the mean product of the deviation from the mean. The covariance for the sampling units is then taken to calculate the regression of yield on ear number.

Table IV. *The analysis of the covariance.*

	Degrees of freedom	Product of the deviation	Estimated covariance
Between sub-plots	5	179·0990	35·8198
Between sampling units	210	9970·4167	47·4782
Within sampling units	648	34434·5000	53·1397
Total	863	44584·0157	51·6617

The value of the regression coefficient of yield on ear number is given by the expression 47·4784 ÷ 72·4659, *i.e.* 0·65518. The yield and the ear number relationship is given by the equation $Y = 4{\cdot}819 + 0{\cdot}655\,x$ where Y is the yield and x the number of ears.

The significance of the linear regression equation can be tested in the following way. 210 degrees of freedom for the variation between the sampling units can be split up into two parts: 1 degree of freedom for the regression line and the remaining 209 for the deviation from the regression line. The sum of squares due to the regression is obtained by multiplying the covariance for the sampling units with the regression coefficient. The analysis is given below:

Due to...	Degrees of freedom	Sum of squares	Mean square
Regression	1	6532·4244	6532·4244
Deviation from the regression	209	5710·1468	27·3212
Total	210	12242·5712	58·2980

More than 50 per cent. of the variation in the yield has been accounted for by fitting a regression line for yield on ear number. That the precision for the prediction of yield when the number of ears is known can be greatly increased is clearly demonstrated in this study. The value of the correlation coefficient is found to be 0·7305.

SUMMARY.

(1) The edge rows give significantly higher yields than the inside rows, indicating thereby the inadvisability of using edge rows in yield trials.

(2) The variation between rows is very much greater than within rows. Different parts of the same drill row should therefore not be

regarded as subject to independent error. The present investigation emphatically confirms Clapham's conclusions on this point.

(3) A slight advantage may be gained by the subdivision of the area to be sampled, without additional labour.

(4) In order to study the effect of the structure of a sampling unit of given size five types of unit have been examined. Of these, method (1), in which the "sampling unit" consists of four parallel half-metre lengths on adjacent rows, appears to be the most precise, and may be recommended on the basis of this trial. The half-metres within such sampling units appear to be negatively rather than positively correlated, and a significantly lower sampling error is obtained in consequence.

(5) Effect of competition between the rows is suggested as the probable explanation for the smaller variation between the sampling units than within them in method (1). Similar analysis on the ear number for the same method, moreover, showed that variation within sampling units was significantly less than the variation between sampling units. This is regarded as additional evidence that there is a competition effect in samples obtained by method (1).

(6) Eighteen such complex units amounting to 36 metres of drill from one-fortieth acre plots would give about 5 per cent. sampling error.

(7) A significant correlation of 0·73 between yield and ear number is obtained. This fact can be used to obtain increased precision for the prediction of yield when the number of ears is known.

In conclusion, I wish to thank Dr R. A. Fisher for his valuable advice and criticism and Mr D. J. Watson for his help in collecting the data.

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Appendix I. *Yield of grain in grams per half-metre.*

Row	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	Total weight of 16 half-metres
1	62·5	44·0	48·0	43·5	40·0	34·5	27·0	24·0	38·0	29·5	14·5	32·0	21·0	11·0	14·5	48·0	532·0
2	25·0	22·0	10·5	12·5	12·0	14·5	19·5	10·0	15·0	9·0	15·5	15·5	15·5	13·0	13·0	33·5	256·0
3	20·5	11·5	21·0	18·5	15·5	12·0	8·0	11·0	10·5	14·0	11·5	13·0	14·5	18·5	11·0	11·0	222·0
4	29·5	17·5	8·5	12·0	5·5	7·5	24·0	14·5	18·5	7·5	14·0	13·5	14·5	17·0	21·0	20·5	245·5
5	24·0	6·5	15·0	16·0	19·0	13·5	22·0	8·0	36·0	19·5	7·0	13·5	15·0	14·5	15·5	16·0	261·0
6	29·5	25·0	8·0	11·0	10·0	21·0	10·5	26·0	18·0	10·0	16·0	9·5	11·0	7·5	17·0	18·5	248·5
7	15·0	10·5	17·0	8·0	12·0	8·0	10·5	13·5	13·0	11·5	9·0	15·0	8·5	6·0	20·5	7·0	185·0
8	22·5	12·0	10·5	12·5	10·0	14·0	26·0	13·0	28·5	10·5	18·5	7·5	22·5	11·5	15·0	15·0	249·5
9	29·5	20·5	10·0	9·0	14·5	17·0	8·5	11·0	14·5	17·0	8·5	11·0	6·0	15·0	22·0	20·0	234·0
10	23·5	41·0	13·5	11·5	7·0	10·5	12·0	12·0	28·5	13·0	12·0	13·5	10·5	20·5	17·5	18·5	265·0
11	28·5	22·5	14·5	19·0	12·0	15·5	19·5	13·0	19·0	12·5	13·5	9·0	16·0	12·0	17·0	25·0	268·5
12	21·0	32·5	19·5	7·0	11·5	11·5	6·0	9·0	17·0	10·5	11·5	15·0	22·5	19·5	3·0	23·5	240·5
13	19·5	27·5	27·5	10·5	19·5	15·0	14·0	15·0	23·0	32·0	31·5	26·0	20·5	19·5	30·0	21·5	352·5
14	34·0	31·0	18·5	24·5	7·0	18·0	16·0	22·0	10·5	23·0	14·0	7·0	27·0	4·5	23·5	11·0	291·5
15	19·0	12·0	19·5	14·0	8·5	11·0	14·0	17·5	8·0	13·5	17·0	21·5	11·0	28·5	10·5	16·5	242·0
16	15·0	16·5	25·5	16·5	12·0	15·0	12·0	28·5	27·5	19·5	24·5	27·0	16·5	26·0	10·5	21·0	313·5
17	15·5	26·5	14·5	11·0	18·0	17·0	22·0	9·0	19·5	13·5	6·5	26·0	23·0	10·5	12·5	13·5	258·5
18	24·5	16·0	30·0	17·0	21·0	17·5	3·5	21·5	11·5	30·0	30·0	18·5	22·0	9·0	40·5	32·0	344·5
19	6·0	11·5	11·0	6·0	11·0	8·0	23·5	12·5	7·5	27·0	23·0	10·0	14·0	23·5	13·5	11·5	219·5
20	29·0	19·0	20·5	22·0	11·0	10·0	10·0	5·5	21·5	30·0	23·0	27·5	30·0	30·5	15·0	12·0	316·5
21	31·0	17·0	18·0	13·0	21·0	17·0	8·0	24·0	15·5	15·5	19·5	21·5	13·5	19·0	11·0	27·0	291·5
22	29·5	30·5	27·0	20·0	18·0	13·0	21·0	26·5	10·5	33·0	30·5	17·0	24·5	23·5	20·0	19·0	363·5
23	25·5	21·0	23·5	15·5	10·0	16·5	20·0	12·5	35·5	34·0	26·5	37·5	39·0	11·5	15·0	13·0	356·5
24	34·5	24·0	9·0	7·5	28·5	15·5	6·5	10·5	21·5	4·0	6·0	16·0	26·5	14·0	17·5	18·0	259·5
25	24·5	23·0	4·0	18·5	20·5	14·5	17·5	15·5	7·0	11·5	23·0	22·5	33·5	7·0	15·0	15·0	272·5
26	34·5	39·5	21·0	12·0	9·0	12·0	19·0	12·5	23·5	28·0	14·0	32·5	32·0	6·0	27·5	27·5	350·5
27	55·0	20·5	24·5	25·5	20·0	19·5	23·5	9·0	16·0	17·0	13·5	17·0	16·5	24·0	11·0	17·5	330·0
28	19·5	42·0	16·0	3·0	21·0	6·0	11·0	22·5	25·0	13·5	5·0	30·0	9·5	19·5	13·0	2·0	258·5
29	39·0	19·0	17·0	41·0	14·0	21·0	23·5	17·5	17·0	34·0	22·0	20·0	7·5	19·5	34·0	28·5	374·5
30	28·0	23·5	32·0	17·0	38·5	30·5	23·0	12·5	25·5	12·5	12·0	16·0	30·0	24·0	17·5	34·0	376·5
31	11·5	22·5	25·5	26·5	22·0	29·5	11·5	15·0	20·0	11·5	26·5	21·0	31·5	29·5	16·5	26·0	346·5
32	20·0	18·0	28·0	18·0	30·0	1·5	25·0	11·0	26·5	17·5	21·5	12·5	9·5	11·5	18·0	10·5	279·0
33	16·5	12·5	14·5	19·5	17·0	21·0	11·0	17·5	15·0	22·0	9·0	20·0	8·0	32·5	17·0	17·5	270·5
34	19·5	19·0	18·0	41·0	10·0	16·5	10·5	16·0	13·0	24·0	22·0	40·5	32·0	12·5	22·0	32·5	349·0
35	25·0	11·5	14·0	15·0	13·0	21·0	16·5	23·5	16·5	20·0	12·5	22·0	9·0	29·0	32·0	34·5	315·0
36	27·5	20·5	7·5	17·5	19·0	14·0	15·5	8·0	28·5	21·0	10·5	22·0	29·0	15·0	12·5	25·0	293·0
37	11·0	21·0	17·0	22·5	26·0	14·0	11·5	12·5	12·0	24·5	30·0	31·0	22·5	21·5	16·5	33·5	327·0
38	7·5	5·5	4·0	1·0	10·5	18·5	2·5	3·0	17·0	16·5	10·5	11·5	9·5	10·5	17·5	4·0	149·5
39	49·5	27·5	19·5	28·0	33·0	20·5	30·0	27·5	18·0	15·5	24·5	33·5	40·0	29·5	17·5	26·5	440·5
40	22·5	24·0	10·5	9·5	30·0	19·5	7·5	16·5	25·5	20·0	11·5	12·5	27·5	1·0	11·5	12·0	261·5

Appendix I (*continued*).

Row	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	Total weight of 16 half-metres
41	17·0	20·5	22·0	17·5	14·0	28·0	25·0	7·5	10·0	19·5	14·5	15·0	21·5	8·5	17·0	17·5	275·0
42	39·5	15·0	17·0	18·5	23·0	18·0	6·0	26·5	25·5	7·5	15·0	15·5	27·0	18·5	26·0	29·5	328·0
43	25·0	26·0	15·5	20·0	17·5	22·5	6·5	20·0	13·5	19·0	15·0	18·5	29·0	8·0	10·5	21·0	287·5
44	19·0	29·5	24·0	20·0	15·5	25·0	15·5	16·0	23·0	18·0	30·5	15·0	25·0	31·5	10·0	11·5	329·0
45	10·0	24·0	22·0	23·5	20·0	13·5	20·0	27·0	8·5	8·0	15·5	15·5	13·5	11·5	13·0	4·5	250·0
46	17·0	19·0	12·5	13·0	16·5	33·5	5·0	8·5	23·0	12·5	20·5	15·5	19·0	10·0	19·5	35·5	280·5
47	19·5	25·0	11·0	10·0	20·5	12·5	32·0	28·0	11·0	24·0	8·5	13·0	15·5	18·5	15·5	32·0	296·5
48	24·5	28·0	12·0	20·0	25·0	38·5	13·0	15·5	19·0	12·5	24·0	26·5	21·0	9·0	28·0	21·0	337·5
49	23·5	31·0	24·0	25·0	26·5	16·5	16·5	21·5	16·5	18·5	18·0	13·5	11·5	23·5	25·0	6·5	317·5
50	35·5	18·0	30·0	21·5	18·5	36·0	29·0	15·5	21·5	7·0	13·0	30·0	31·0	23·0	20·0	29·5	379·0
51	12·5	0·0	9·0	13·0	14·0	6·0	13·0	21·0	0·0	4·0	17·5	8·5	0·0	12·5	24·5	2·5	158·0
52	11·5	23·0	16·0	14·5	24·0	27·5	19·5	15·5	13·0	14·0	9·5	21·0	27·5	8·0	10·5	22·0	277·0
53	39·0	32·0	11·5	15·0	6·0	10·0	14·5	13·0	22·5	18·0	11·0	9·0	12·5	16·5	15·5	14·5	260·5
54	6·0	3·0	10·0	21·5	30·0	14·5	13·0	10·0	7·5	3·0	8·0	18·5	15·0	14·5	15·5	7·0	197·0
55	26·5	5·5	6·0	23·0	10·0	3·0	9·0	19·5	4·5	17·5	15·0	16·5	8·0	23·0	26·0	18·0	231·0
56	12·0	55·5	27·5	14·5	21·0	26·0	18·0	7·0	18·0	7·0	9·5	15·5	15·0	23·5	10·0	7·0	287·0
57	54·5	19·0	14·0	20·0	43·0	35·5	24·5	24·0	17·0	11·0	17·0	10·0	15·0	8·0	6·0	22·0	340·5
58	25·5	27·5	37·5	7·5	8·5	14·0	2·5	7·5	15·5	18·0	14·0	18·0	25·5	40·0	29·0	23·5	314·0
59	25·0	13·5	12·5	28·5	12·0	16·0	20·0	16·5	29·5	0·5	2·5	14·0	15·0	11·5	0·5	12·0	229·5
60	18·0	27·5	26·0	20·0	11·0	20·5	13·5	0·0	13·0	14·5	17·5	10·5	16·0	17·5	24·5	23·5	273·5
61	25·5	16·5	26·5	20·0	33·5	20·0	37·5	20·5	21·0	14·5	28·5	21·0	9·0	0·0	14·5	13·0	321·5
62	36·0	28·0	15·5	22·5	16·0	25·0	19·0	15·5	16·5	17·5	9·5	4·0	26·0	24·0	2·0	22·0	299·0
63	15·0	3·0	20·5	11·5	7·0	10·0	18·0	31·0	17·5	25·5	16·5	5·5	9·0	8·5	26·5	35·5	260·5
64	30·0	11·0	5·5	16·0	21·0	19·0	4·0	7·0	13·0	1·5	3·5	9·5	23·0	21·0	6·5	18·5	210·0
65	35·0	30·5	30·5	41·5	22·5	21·0	23·5	23·5	25·0	37·5	17·5	45·0	23·5	20·5	21·0	36·5	454·5
66	24·0	17·0	20·5	39·5	18·5	15·5	17·0	12·0	27·0	16·5	17·5	9·0	33·0	30·5	20·5	27·5	345·5
67	37·5	16·0	21·5	20·0	31·5	20·0	18·0	23·0	16·0	27·0	15·0	27·5	34·0	11·0	26·0	30·5	374·5
68	28·0	31·5	26·0	24·5	24·0	12·0	22·0	18·5	34·0	15·0	20·5	16·0	16·0	22·5	27·0	21·5	359·0
69	12·5	20·5	14·5	22·0	9·5	17·0	28·0	15·5	17·0	22·5	7·0	24·5	38·5	19·0	14·5	31·0	313·5
70	29·0	15·5	22·5	12·0	29·5	27·5	22·0	28·0	23·5	27·5	33·0	23·0	11·0	26·5	22·5	4·5	357·5
71	23·5	19·0	22·0	20·5	19·0	24·5	19·0	28·0	19·0	27·0	6·5	28·5	30·5	30·0	23·5	25·0	365·5
72	21·0	23·5	10·5	15·0	33·0	16·0	9·0	19·0	21·0	11·0	21·0	17·0	16·0	14·0	23·5	18·5	289·0
73	38·5	23·0	28·5	20·0	17·5	28·0	16·0	27·0	17·5	28·5	24·5	10·5	27·5	29·0	20·0	3·0	359·0
74	12·0	18·0	13·0	23·5	9·5	18·0	31·5	24·5	20·0	23·5	18·0	6·5	10·5	17·0	12·5	38·5	296·5
75	18·5	16·0	26·0	20·0	19·0	15·5	19·5	27·5	31·0	19·0	19·0	20·0	20·5	27·5	27·0	20·5	346·5
76	23·5	22·5	13·0	12·0	22·0	16·5	13·5	8·5	17·5	20·0	14·0	23·5	22·5	3·5	14·0	12·5	259·0
77	18·0	30·0	9·0	17·5	24·5	15·5	33·5	30·0	17·0	32·0	11·0	0·0	30·0	13·0	8·0	9·5	298·5
78	31·5	10·0	43·5	27·0	12·0	3·0	11·5	20·5	20·0	23·0	22·0	40·0	8·5	15·0	17·0	12·0	316·5
79	30·0	20·0	11·5	28·5	0·0	23·0	12·0	18·5	20·0	22·5	13·0	32·5	20·5	5·0	15·5	4·5	277·0
80	47·5	49·0	29·0	58·0	38·0	41·0	29·5	20·5	29·5	50·0	51·5	21·5	20·5	39·0	22·5	3·0	550·0
Total	2022·5	1730·5	1483·0	1511·5	1472·0	1437·5	1343·0	1348·5	1509·5	1464·0	1336·5	1492·5	1586·0	1392·0	1416·5	1567·0	24112·5

Appendix II. *Number of ears per half-metre.*

Row	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	Total number in 16 half-metres
1	39	29	40	29	22	22	23	18	26	24	15	22	15	5	8	29	366
2	24	19	19	25	20	19	23	13	14	10	21	18	11	7	11	25	279
3	25	10	27	20	19	20	13	15	11	16	14	17	14	23	11	9	264
4	29	26	21	19	11	20	39	23	28	15	26	20	26	26	18	23	370
5	27	8	16	30	21	23	34	14	41	19	11	19	20	18	12	15	328
6	29	34	23	31	24	24	19	30	15	18	18	14	20	10	19	22	350
7	9	10	23	25	19	13	15	22	24	11	22	33	13	6	24	9	278
8	22	16	25	26	16	31	35	18	44	17	33	11	28	16	22	14	374
9	25	21	20	14	26	20	15	12	17	20	17	14	5	16	19	21	282
10	23	35	18	30	9	12	25	15	32	12	20	21	14	12	17	15	310
11	28	28	25	32	18	21	22	16	20	20	26	20	23	19	17	20	355
12	19	31	22	9	10	14	13	19	22	11	20	19	28	20	4	23	284
13	21	33	26	16	29	30	18	17	27	26	32	21	24	18	27	21	386
14	27	30	29	26	16	25	21	16	8	18	20	4	23	6	26	17	312
15	32	15	37	15	16	20	16	17	6	14	20	24	11	25	13	18	299
16	19	15	23	22	16	16	14	29	29	22	27	30	18	29	13	28	350
17	22	27	18	16	28	19	20	9	19	20	6	29	20	12	20	16	301
18	38	20	28	23	28	22	6	19	19	25	28	19	18	11	34	32	370
19	12	15	18	9	23	8	27	19	13	30	21	22	13	27	23	16	296
20	36	26	29	22	25	15	10	10	23	22	29	32	20	28	23	15	365
21	26	25	29	20	25	24	12	23	11	20	16	23	15	18	18	30	335
22	38	27	33	19	28	26	31	30	7	26	42	17	29	26	20	24	423
23	25	41	27	15	10	26	31	15	27	29	30	30	31	17	17	24	395
24	22	29	11	8	24	24	16	12	19	3	6	17	24	15	38	29	297
25	20	34	3	23	23	23	23	16	8	10	18	18	27	8	17	28	299
26	24	22	22	19	18	22	29	19	30	28	21	33	34	7	30	35	393
27	40	20	30	20	23	21	26	13	15	17	19	11	14	22	12	12	315
28	15	41	17	7	23	7	11	25	24	18	8	26	11	21	15	3	272
29	25	23	25	35	14	27	28	30	24	31	22	17	11	20	31	17	380
30	23	30	32	23	36	34	31	19	26	16	14	12	26	31	23	26	402
31	15	24	30	29	29	32	18	19	25	16	35	18	30	31	20	31	402
32	30	26	34	21	39	4	28	20	33	28	32	14	10	10	19	12	360
33	14	23	17	20	19	25	17	21	19	23	19	21	10	34	15	16	313
34	18	23	31	36	26	22	16	19	17	30	23	43	26	18	22	28	398
35	24	15	14	17	24	26	23	23	18	30	14	20	9	22	27	31	337
36	23	25	15	20	24	24	33	13	23	29	20	20	27	18	20	28	362
37	15	19	23	27	26	32	22	28	16	31	28	29	23	22	21	35	397
38	11	7	4	3	10	21	7	7	18	24	17	13	10	13	31	5	201
39	33	28	17	32	21	25	28	32	20	21	27	27	33	31	23	26	424
40	25	25	17	13	32	19	8	18	27	19	15	9	24	1	9	11	272

Appendix II (*continued*).

Row	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	Total number in 16 half-metres
41	16	17	24	26	18	30	30	16	13	19	17	20	26	9	23	17	321
42	35	13	25	16	28	26	10	30	35	10	24	22	26	20	24	24	368
43	17	18	20	20	21	27	10	16	12	25	16	21	25	7	12	23	290
44	22	22	27	23	24	27	12	16	23	17	30	15	23	23	8	12	324
45	15	21	22	28	31	14	22	22	11	6	19	17	14	11	16	3	272
46	23	20	11	22	23	32	6	9	23	11	29	12	22	10	17	29	299
47	22	27	14	15	32	9	21	28	17	20	6	12	16	13	16	26	294
48	31	28	14	21	26	30	18	17	23	16	28	23	19	9	26	21	350
49	28	26	27	33	24	21	15	22	20	23	21	12	13	25	23	9	342
50	34	18	25	27	24	29	34	15	17	15	16	26	37	18	15	28	378
51	12	0	11	24	18	6	11	18	0	6	20	8	0	12	24	2	172
52	16	21	14	27	25	34	20	18	16	14	8	17	28	5	10	18	291
53	31	23	10	22	6	13	21	17	23	20	11	10	10	14	13	13	257
54	8	3	8	25	26	10	7	7	13	4	10	18	11	10	17	7	184
55	28	8	7	30	7	3	10	16	4	19	17	22	8	21	26	17	243
56	21	38	22	15	23	17	22	10	20	14	8	20	15	19	9	6	279
57	49	14	17	21	26	28	22	22	21	8	17	10	12	7	9	23	306
58	25	23	35	7	9	11	3	8	14	17	14	25	23	36	34	24	308
59	23	9	9	18	8	11	18	14	24	1	2	13	9	10	1	9	179
60	14	24	23	12	11	19	11	0	20	12	24	12	14	20	20	17	253
61	20	12	23	16	29	21	25	18	20	12	23	19	11	0	18	9	276
62	23	25	10	15	17	22	19	17	16	17	12	5	21	28	5	22	274
63	16	6	13	8	7	11	20	38	15	24	25	6	10	15	25	35	274
64	25	14	8	14	27	21	5	10	16	2	4	10	19	15	11	21	222
65	35	40	33	40	28	24	24	29	30	37	23	35	24	16	24	37	479
66	22	18	35	43	22	17	21	16	34	19	23	10	36	27	26	32	401
67	34	13	30	21	33	19	24	24	26	29	25	26	37	13	29	42	425
68	36	30	23	29	26	17	21	21	40	19	18	22	20	22	26	22	392
69	10	21	15	22	8	24	33	25	18	27	10	27	40	20	19	29	348
70	26	18	22	21	27	31	25	27	27	32	40	27	11	20	25	6	385
71	27	19	28	25	24	32	26	33	23	23	7	33	30	22	24	21	397
72	15	24	16	12	40	17	10	30	19	12	20	18	12	12	25	15	297
73	31	17	27	25	17	32	12	30	19	28	25	13	24	25	24	2	351
74	18	15	19	21	10	28	30	27	23	20	21	7	12	15	9	37	312
75	22	14	31	18	21	19	24	29	29	22	17	22	16	26	22	19	351
76	24	14	15	13	25	14	15	9	20	19	12	21	25	8	17	9	260
77	19	23	11	15	30	14	35	28	17	30	10	0	29	10	6	8	285
78	22	11	36	33	11	3	13	23	18	27	20	35	5	13	18	17	305
79	19	13	9	29	0	23	13	17	14	18	14	25	17	4	15	6	236
80	32	31	22	38	24	29	26	14	25	30	31	15	12	23	19	3	374
Total	1918	1706	1709	1736	1706	1673	1590	1539	1643	1543	1569	1538	1550	1352	1519	1559	25850

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THE EVOLUTION OF DOMINANCE

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I. EARLY SPECULATIONS ON DOMINANCE.

IT was recognised early in the discussion of genetic phenomena that the two great facts demonstrated by Mendel's breeding experiments in the garden pea (*Pisum*), segregation and dominance, had very different bearings upon our interpretation of genetic and evolutionary phenomena. Segregation, due to the particulate nature of the hereditary elements, was a primary and essential fact in the hereditary mechanism; dominance was an additional fact, not essential for explaining the hereditary mechanism, but rather an obstacle to its understanding, as is shown by the frequent use of the blue Andalusian fowl, a heterozygote quite unlike either the black or the flecked white homozygote, in explaining the Mendelian theory. Nevertheless, its immediate practical importance appears from the universal recognition of the 3 : 1 ratio, rather than the genetic 1 : 2 : 1 ratio, in the offspring of selfed or interbred heterozygotes. And, just because it is a fact logically independent of the factorial system of inheritance, it has exerted a very important influence on the evolutionary speculations of the early writers.

The first general statements respecting dominance referred dominance to certain characters rather than to certain genes. Thus, numerous cases in which the recessive was white, or of a lighter colour, or of a simpler pigmentation, than the dominant, suggested, in spite of exceptions, a general notion that pigmentation is dominant to absence of, or less, pigmentation. To this stage in genetical concepts belong such statements as that tallness is dominant to shortness, not in reference to a particular pair of allelomorphic genes studied by Mendel in *Pisum*, but as a biological principle already proved in *Pisum*, the general applicability of which would be explored by further research. While dominance was thought of in this way, the available examples formed a general impression that the dominant characters were more positive, or complete, than the recessive characters, an idea which seems to have influenced later speculations a good deal.

Facts, however, soon headed off speculation as to the dominance of characters, as opposed to that of particular genes. Both dominant and recessive whites were found in fowls, both dominant and recessive piebalds in mice. Breeding tests showed that the different Mendelian factors involved had no genetic connection; they merely manifested themselves by the same or similar somatic contrasts. Nevertheless, dominance or recessiveness were evidently not assigned at haphazard; knowing the effect of a factor, one could make a shrewd guess as to which phenotype would be the dominant. On what were such guesses based? What rules did the incidence of dominance appear to obey? Two criteria were prominent:

(i) The recessive was often defective. Deficiency of pigmentation, of chlorophyll in plants, or of the banded structure of the hairs in the wild or "agouti" pattern in rodents, were clearly of this type; also such structural defectiveness as is shown by the inner ears of "waltzing" mice, and in many cases of malformation so gross as to be classed as monstrous. The defects of the wings and bristles of *Drosophila* added a number to the list of recessive defects.

(ii) Novelties were usually recessive. In such a plant as the Sweet Pea (*Lathyrus odoratus*), the Mendelian analysis of the numerous cultural varieties showed in every case that the characteristic of the dominant genotype was that of the presumptive wild ancestor. With the study in *Drosophila* of mutants certainly arising in culture, it appeared clearly that these were generally recessive to the genes of the wild type of fly; and this in spite of the fact that dominants were more easily and more quickly detected, and were much valued by the geneticists.

A theory which connected some of these facts, and, in spite of its inherent difficulties, has, in the absence of satisfactory alternatives, exerted a considerable influence, was put forward by Bateson and Punnett, as the "Presence and Absence Hypothesis." According to this view the recessive genotype was totally lacking in a gene which was present in the dominant genotype. Dominance was due to the supposed fact that a single gene in the zygote was capable of exerting the same influence as if the normal pair had been present. The recessive genotype, lacking these genes altogether, displayed its genetic deficiency by a visible somatic defectiveness. Mutation consisted, usually if not always, of the loss of a pre-existent gene. The evolutionary consequence was boldly drawn by Bateson that the genetic outfit of existing animals and plants was a residue or remainder of the complete genetic outfit of their primordial ancestors. Evolution consisted in "unpacking" the germ plasm, all the possibilities of which had been present from the first. A somewhat comprehensive process of creation was relegated to the distant past.

The fact that some mutations were at least partially dominant was met by the ingenious, but dangerous, hypothesis that they were due to the loss of "inhibitors." In cases where dominance is absent or incomplete, it was supposed that for some special reason the single gene could not produce the same effects as a homologous pair. These special explanations of the rarer types of relationship damaged a good deal the general plausibility of the theory; and in the case of the dominant characteristics of several breeds of poultry, to which the wild type is recessive, Punnett was willing to consider the view that "something new had been added."

The "Presence and Absence Hypothesis" became untenable when it was shown that not two only but three or more different allelomorphs often existed belonging to the same Mendelian factor. Not more than one of these could be postulated as an "absence," and the admission, in some cases, of two different kinds of "presence," one completely dominant to the other, greatly strengthened the view that in other cases also the recessive gene was not a mere "absence." That mutations do not consist simply of losses was decisively contradicted by the occurrence of reverse mutations, and though for a time many of the earlier reports of these could be explained away, their occurrence has recently been fully demonstrated by Patterson and Muller (1930) by the use of X-rays. If a mutation is a "loss," its reversal must be counted a "gain," and once such gains are admitted the ground for supposing that mutations in general are frequently of so simple a character as a mere loss falls away.

In view of these facts, the original presence and absence hypothesis has been replaced in practice by the more tenable, though perhaps too simple, view that a series of multiple allelomorphs may differ only quantitatively in respect of some one physiological or biochemical function, so that in such a case as the white-eye series in *Drosophila*, we should think of the wild red-eyed fly as containing fully active genes, and the various mutant allelomorphs as containing, in order of the depth of pigmentation, genes of the same kind, only less and less active. That this may in some cases really be true is strongly suggested by the fact that a mutation such as Notch 8, which may be regarded on good evidence as really due to the absence of a small tract of chromatin, containing the white eye locus, behaves in conjunction with the allelomorphs of white eye as though it contained the most extreme member of the series. The supposed connection, however, between such inactivation and recessiveness has been challenged by Ford (1930), who points out that this very series are not recessive to the wild type in respect of certain internal characters.

II. GENERAL EVIDENCE AS TO DOMINANCE.

Setting aside the suppositions:

(i) that Mendelian allelomorphs are always pairs which can be formally identified as the presence or absence of something;

(ii) that mutations are always or usually merely losses or inactivations of nuclear material; and

(iii) that such loss or inactivation is in itself a sufficient cause of recessiveness; we are in a position to reconsider what the evidence available really has to tell us as to the incidence, and causes, of the phenomenon of dominance.

It should be emphasised at the outset that dominance is an observational fact, involving a comparison of the somatic characters of three different genotypes, two homozygotes and the heterozygote formed by crossing them. In such a comparison it is evident that the three genotypes compared should, properly, differ only in the one factor under consideration, otherwise the effects of other factors, or of dominance in other factors, will be involved. If the heterozygote is found to be indistinguishable from one of the homozygotes, that homozygote is said to be completely dominant and the other completely recessive. If the heterozygote were equally different from both homozygotes, dominance would be absent, and neither gene should be said to be dominant to its allelomorph. Between these extreme cases we may recognise cases of incomplete dominance in which the heterozygote resembles one homozygote perceptibly more than the other, while resembling neither completely. The phenomenon is not a genetic one in the sense that further experimental breeding can throw light on an ambiguous observation, but is purely somatic and observational. In view of the fact that all three genotypes are usually variable, increased precision may be obtained by observing groups of the three genotypes to be compared, and observations made in this way are susceptible to any degree of biometrical refinement.

The terms as above defined have not been used very strictly in the literature. For example, many lethal genes have visible effects; that is to say, one homozygote is inviable while the heterozygote differs visibly from the viable homozygote. Such genes are usually spoken of as dominants, or sometimes as lethal dominants. They should, I think, be called visible lethals in contradistinction to the recessive lethals which have no visible effect when heterozygous. Even the elusive class of lethal genes which cause death both in the homozygous and the heterozygous condition should, I suggest, only be regarded as dominant lethals if the two lethal phases are known to be similar up to the time of death. Again, where, as in *Drosophila*, a definite wild type is available as a basis for comparison, it is usual to speak of all mutants which are not completely recessive as dominants, merely to indicate that the heterozygote is distinguishable from the wild type, without reference to whether the heterozygote is more like to the wild fly, or to the homozygous mutant.

The factors, the behaviour of which has been studied by experimental breeding, fall broadly into three classes:

(*a*) Mutations arising in culture, which in the past have been principally available in non-domesticated animals such as the fruit flies *Drosophila* and the shrimp *Gammarus chevreuxi*, which are bred as convenient genetic material. Such mutations, induced by X-rays, will, it is to be supposed, soon become abundantly available in many other animals and plants.

(*b*) Differences between different varieties of domesticated animals and plants, which have originated presumably by mutations in the past, but whose effects have often been modified, by combination with other factors, to a large extent in the course of human selection.

(*c*) Differences between the various forms of species which are polymorphic in nature.

The evidence as to the incidence of dominance provided by these three classes is naturally very different in character, and it was especially the simplicity of the rules followed by the mutants that first led me to speculate on the subject. The conditions for studying dominance are here much better than with domesticated races, for the mutant will usually differ from other members of the stock in which it arose only in one factor affecting the characteristics to be observed.

The great majority of mutations in *Drosophila* are lethals, nearly all completely recessive, though a minority have visible effects. With respect to the non-lethal mutations in *Drosophila melanogaster* I found, on classifying the 221 different mutations reported by Morgan, Bridges and Sturtevant (1925), that 208 were classified as recessives and thirteen as dominants. The recessives were in fact sixteen times as numerous as those classed as dominant, and of the thirteen cases of dominance recorded the dominance was in every case incomplete; that is to say, the homozygous mutants were always distinguishable from the heterozygotes, which, indeed, showed all degrees of intermediacy between the wild type and the homozygous mutant.

The second fact of importance, which seems to be general in the *Drosophila* mutations, is that in the many cases of multiple allelomorphism, where several distinguishable mutant genes have arisen from the same gene of the wild fly, the wild-type gene is completely dominant to all its mutants, while these give with each other heterozygotes of an intermediate character. Dominance is, in fact, conspicuously absent from the genes of such allelomorphic series with the single exception of the particular allelomorph which prevails in the wild population.

The phenomenon of recessiveness to the wild type is also very generally observable in the domesticated races of animals and plants, though crosses between different varieties of these provide generally far less suitable material for the study of dominance than do the mutants that arise in culture. It is particularly fortunate therefore that a very thorough study of the albino series of multiple allelomorphs, which occurs in most rodents, has been carried out by Sewall Wright (1925) in the guinea-pig. Using five allelomorphs of this series, Wright bred the five homozygous and the ten heterozygous types, in which they may be combined, in sufficient numbers to study both the average depth of pigmentation of the red and the black parts of the animal and its variability between different individuals of the same genotype. The results of this investigation were perfectly clear-cut and decisive. The five homozygous genotypes were all easily distinguishable. Of the ten heterozygotes four, which contained the wild gene, were indistinguishable from the wild homozygote, showing its full pigmentation in both respects. The remaining six heterozygotes, containing two different mutant genes, were, in each case, intermediate in appearance between the two corresponding homozygotes.

This remarkable, and uniform, behaviour of the allelomorphic series, supplies, I believe, a direct clue to the interpretation of dominance phenomena generally, for if we are to assume that in the course of evolutionary change individual genes have been replaced, for whatever reasons, by mutant allelomorphs, it is evident that the member of an allelomorphic series which was prevalent in the wild population at one stage must have been in the past, and, by reverse mutation, might come to be in the future a mutant member of the same series. The rule that the wild-type gene must be dominant to all its competitors could only continue to hold if, in the course of evolutionary change, it *became* dominant to them. The cause of dominance should on this view be sought as a by-product of the causes which lead one gene rather than its allelomorphs to prevail in the wild population. It becomes necessary at this point to enquire into the possibility of the modification of dominance by selective agencies.

III. SELECTIVE MODIFICATION OF THE EFFECTS OF MENDELIAN FACTORS.

Evidence of the selective modification of the effects of Mendelian factors is, in fact, when looked for, exceedingly abundant. Anyone who has bred, for example, mice, for genetic purposes, knows with what contempt his productions would be viewed by the judge at a fancier's show. He knows that the show "Dutch" mice are recessive pied, but their shoulder patches have been suppressed, the head markings have been confined to two beautifully symmetrical ovals round the eyes and ears, and the rump markings confined to a broad straight band across the body. The ideal prize-winner may be a rare product of its own genotype, and probably has to be heterozygous for one or more special factors. Its genetic production would certainly require a very detailed study of the factors which modify the pied pattern. And the same is true of the show product in nearly all the fancy breeds of animals; the geneticist can only recognise the gross differences caused by those factors whose effect is pronounced enough for him to study. The showman, by selecting a multitude of modifying factors, has modified the crude genetic type almost out of recognition.

More immediately to the point is the spontaneous modification which has frequently been observed in mutant strains soon after their isolation. Several workers with *Drosophila* have reported cases in which a mutation, having well-marked bodily effects, has been set aside to be bred in stock bottles until it could be used. After several generations of breeding in this way the mutant has appeared to be less distinctive and more normal than it was at first; it seems to have reverted somewhat towards the wild type. Such mutations often show, from the first, some degree of reduced viability, besides some variability in the intensity with which the mutant character is manifested, or, in other words, in the violence of their reaction to the mutant gene. The modification of the mutant genotype which has taken place is apparently due to the selection of modifying factors, which mitigate to some extent the effect of the mutation. That this is the true explanation has been verified by outcrossing the modified mutant to unrelated wild flies, and recovering the mutant by inbreeding the offspring. The mutant type so recovered has been partially de-modified, and shows a return towards the extreme condition originally observed.

A very similar case has been reported by Prof. F. E. Weiss, of a mutant nasturtium, which had modified leaves and was very sterile. It was in consequence propagated with considerable difficulty, and by the time that a satisfactory strain was obtained the leaves were found to have reverted, to a considerable extent, towards the normal nasturtium form. Mr E. B. Ford also informs me that it is the general rule among the mutants he has studied in *Gammarus* that they have at first an extremely low viability, and that only after considerable modification by natural selection are healthily viable stocks obtained.

It has, of course, long been known that genes which have a pronounced effect in the presence of some other gene may have none in its absence. Terms such as epistatic factors, complementary factors, specific modifiers, etc., have only been introduced in recognition of particular cases of the general fact that the effect of any one genetic substitution depends upon the gene complex, or genetic background, in which the substitution is made. What the particular cases cited demonstrate is that, even in small isolated stocks, a sufficient variety of modifying factors is usually present to produce, in a few generations, a considerable effect upon the appearance of a homozygous mutation subjected to direct selection. It would be astonishing if such selection were in any degree less effective in modifying the appearance of the heterozygote, had the heterozygote been the phase subjected to the selective action.

IV. SELECTIVE ACTION ON THE HETEROZYGOTES OF RECURRENT MUTATIONS.

Now it is certain that many of the *Drosophila* mutations occur with mutation rates of about one in a million, that is to say, that in each generation one in every million of a particular kind of gene undergoes the transformation in question. There is no reason to suppose that the mutation rates in the wild population are, or have been, lower than those observed in culture. As to the length of time during which the same mutation has been occurring, we have direct evidence that the mutations of different species in *Drosophila* are frequently homologous; many mutations therefore certainly antedate the fission of these species. This, moreover, is only a lower limit, for the direct proof cannot be extended beyond the range of species crosses. The close analogy, however, between the different allelomorphic series found in rabbits, rats, mice and guinea-pigs, indicates strongly that the same mutations have here been occurring through a great part of the period occupied by the differentiation of the rodents, and makes it seem less improbable that a mutation such as albinism, which occurs in the most diverse orders of mammals, has been occurring throughout the history of the class. It seems, indeed, impossible to set an upper limit to the antiquity of the oldest mutations which may be now occurring, for a lethal factor causing the death of the zygote in the one-celled stage might, for aught we know, have persisted in occurring throughout the whole history of the metazoa. It is probable, however, that the genetic changes which have brought about the evolutionary transformation of species have been accompanied by corresponding changes in the frequency and kind of the deleterious mutations to which their germplasms are prone, so that, although many mutations are undoubtedly enormously ancient, there is no reason to regard them as more ancient than those morphological features of animals which are regarded as of systematic importance.

If such a mutation has persisted in occurring among the ancestry of an existing population, and has been constantly kept rare by counter-selection, it is a matter of some importance to calculate the average frequency, in each generation of this ancestry, of the heterozygous and of the homozygous mutant; for these frequencies should measure the efficacy of natural selection acting upon the modifying factors, in mitigating the reaction of the existing population to the mutation in question. It appears that the heterozygote will, in such cases, be so enormously more frequent than the homozygote that, except when its viability is within a very minute fraction equivalent to that of the wild type, and except in the case of sex-linked factors, the modification of the homozygous mutant need not be considered. With respect to the heterozygote, the case is very different. Its frequency will be, of course, proportional to the mutation rate, but will depend also greatly upon its viability, or frequency of parenthood. If, for example, this differs from the normal by only one per cent., with a mutation rate of one in a million, the proportion of heterozygotes in each generation in the ancestry of the existing population will have been about one in five thousand. With a viability of ninety per cent. the fraction has fallen to about one in sixty thousand, and at fifty per cent. to one in seven hundred and fifty thousand. These fractions should, in my view, represent the rates of modification of the heterozygotes in nature, in comparison to the rate of modification which could be brought about by selection applied to a population consisting entirely of mutants. Since this, even when applied to homozygotes, is certainly capable of producing noticeable effects in a short period, it appeared to me, and I can see no reason to doubt the soundness of the conclusion, that natural selection of the heterozygotes must be an agency in causing them more and more to resemble the non-mutant homozygotes, acting with a combined intensity and duration which cannot safely be neglected.

Since, however, the efficacy of such minute selective actions has been questioned, not on the ground that the time available is insufficient, but that they would be ineffective however long the time available, and since it is manifestly impossible to prove experimentally that a selective intensity one ten-thousandth of another will really produce the same effect in ten thousand times the time, it will be better, as in the other cases indicative of the modification of dominance, to which this objection does not apply, to follow out the qualitative consequences of the theory, and compare them with such known facts as are relevant.

If we suppose that at its first appearance the mutant heterozygote was intermediate in appearance and viability between the normal form and the mutant homozygote, its subsequent fate would depend greatly upon its initial viability. We have seen that as the viability improves, the intensity of selection is greatly accelerated, consequently those with an initially high viability would have time to become completely normal in appearance, before others, more heavily handicapped at the start, had made any appreciable progress. After the heterozygote has become completely normal, and the mutation in question has become completely recessive, a second process of modification will commence, this time in the homozygote, which as calculation shows (Fisher, 1928 *a*) can now be maintained at a sufficient frequency in the population for this process to be effective. In the case of the homozygote, the initial disadvantage will probably be considerably greater, and the initial rate of improvement enormously slower. Nevertheless, in favourable instances it, too, may attain a high level of viability, in which case it also will probably be modified to an extent which renders it indistinguishable from the normal type. Such factors would, in themselves, necessarily be overlooked, for they produce no visible effects. They may, however, occasionally be brought to light as specific modifiers of other factors which are being studied.

In the case of lethals in which the homozygote invariably perishes, the second stage of modification is of course impossible, and the pause when the heterozygote becomes normal is indefinitely prolonged. Consequently we should expect the greatest number of factors to be accumulated at this stage, and it is noteworthy that by far the most numerous class of mutations occurring in *Drosophila*, either naturally or under the influence of X-rays, are completely recessive lethals. The heterozygotes of lethal genes might be expected to be more heavily handicapped than those of non-lethal genes, and we accordingly have a considerable class of visible or "dominant" lethals. Of the non-lethals, as we have seen, sixteen out of seventeen are classed as recessives, and may be regarded as having completed the first stage of their modification. The few that remain as incomplete dominants might conceivably be mutations of sufficiently recent origin as to have made, as yet, but little progress in modification. The fact that they have appeared in culture does make it probable, however, that their mutation rate is fairly high, and, unless mutation rates can change suddenly, which seems improbable, this in itself would seem to imply that they are probably not recent. The alternative view that they are on the whole somewhat heavily handicapped in respect of viability seems completely to fit this group of cases.

V. THE ABSENCE OF DOMINANCE IN CASES WHERE SELECTION CANNOT BE POSTULATED.

We have seen that a general view of the dominance phenomena exhibited by the mutants in *Drosophila* accords well with the opinion that recurrent mutations having deleterious somatic effects have become gradually recessive to the prevalent wild genes; and that the facts suggest further that the selective action has been sufficiently rapid to have effected a considerable change in the majority of those cases which come under observation. Important evidence is, however, also available from cases in which, in the absence of such counter-selection, dominance is likewise found to be absent, and these classes of observations, which we shall now consider, make it difficult to believe that explanations of dominance, which rely upon special assumptions as to the biochemical situation in the nucleus, can have more than occasional applications.

(*a*) We have already mentioned the absence of dominance which is usual between the different mutant allelomorphs of the same gene. In this case it is evident that although one allelomorph may well be more advantageous than another, the extreme rarity in nature of heterozygotes carrying in the same locus two different mutant genes, and the low probability of such individuals contributing to the ancestry of the existing population, will have precluded the modification of these heterozygous types by natural selection.

(*b*) Mr E. B. Ford (1930) has called attention to the extremely important fact that many of the *Drosophila* mutations such as the white-eye series, and the body-colour mutants "sooty" and "ebony," while recessive in their effects upon the colour of the body and the eyes, are yet not recessive in the minute constant effects which they exert, as was shown by Dobzhansky (1927), upon the shape of the spermatheca. Ford points out that the intensity of selection upon such a character as body colour is probably considerable, as is, with equally high probability, that on the pigmentation of the eyes, but that in the case of a small change in the proportions of an internal organ we have exceptionally good grounds for presuming the absence of selective action. Consequently recessiveness would only have been produced if the genetic changes needed to modify the external characteristics had also modified the internal organs towards the normal shape. It is difficult to reconcile a series of cases of this kind, which, apart from special investigations, would inevitably have been overlooked, with the view that there is anything in the intrinsic nature of mutations, as such, which makes for recessiveness.

(*c*) Many cases are known in which a mutant is completely recessive to its wild allelomorph when these are compared in animals otherwise of the wild type, but in which the recessiveness becomes incomplete when they are compared in the presence of other mutant factors.

G. D. Snell, in a recent summary (1931) of the mutants which have been studied in mice, supplies several instances of this. Thus, in the *albino* series he notes that full colour is normally completely dominant to all other members of the series, but that in the presence of "pink-eye" heterozygotes carrying the albino gene, or even chinchilla, another allelomorph of this series, are appreciably lighter than those homozygous for colour. He further mentions that mice heterozygous for albinism have been reported by more than one worker to develop large patches of white or silvered hair as they grow older, and that when treated at ten to fourteen days of age with just sufficient X-rays to cause the hair to fall, they regenerate a semi-white coat. Again, "*brown*" or "chocolate" is usually entirely recessive to its allelomorph "black," but pink-eyed non-agouti mice heterozygous for brown are distinguishable from those homozygous for black. Still more striking is the effect of "silver"; for silver mice heterozygous for brown show a greatly intensified silvering, the underfur being practically white. Chocolate-silvers, despite the two chocolate genes, are stated to be often darker than the heterozygotes, though distinguishable from them by the colour of the unsilvered hairs. With *recessive pied* the incomplete recessiveness is so frequent that Snell describes it as an imperfectly recessive character. In the presence of the lethal "dominant pied," heterozygotes carrying "recessive pied" are undoubtedly distinguishable by their more restricted pigment; in my own experience, however, litters sired by wild mice on recessive pied mothers show no sign of white spotting; recessive pied seems thus to be completely recessive when examined in conjunction with the wild gene complex.

In *Drosophila* the case of "forked" and "semi-forked" is of interest as showing that a modifier of dominance may be almost without effect upon either homozygote. In the wild fly forked is an ordinary recessive which produces a shortening, twisting and thickening of the bristles. It is sex-linked, so that dominance can only be examined in the female. In the course of Dr Lancefield's experiments with this factor in 1918 the mutant semi-forked was discovered; this mutant has no distinguishable effect upon the homozygous forked females, or upon the forked males. It produces, but rarely, a slight shortening of the bristles in normal flies. Females heterozygous for forked, however, are modified by it into clear intermediates. In this connection we may recall also that the white-eye mutant in *Drosophila*, which, by itself, is a typical recessive, has, when heterozygous, a distinct diluting effect on flies homozygous for any of several other light eye-colour mutants. The fact that genetic combinations can be made up, in which the heterozygote differs from the non-mutant homozygote, is again difficult to reconcile with any general biochemical explanation of dominance, and indicates that we should look for some other explanation of the fact that these genotypes resemble one another so closely, when the effect of the gene is examined against a wild background.

(*d*) A case of very particular interest was brought to my knowledge by Mr J. B. Hutchinson, and arose in the investigations into the genetics of the cotton plant, which Dr S. C. Harland has been carrying out in Trinidad. The interest of the case in the present connection has led to a number of further experiments, and to consequent delay in publication, so that the original facts I can now give are subject to the confirmation or reinterpretation afforded by the later experiments.

It appears that a mutant form known as "Crinkled Dwarf," which occurs in the Sea Island cottons, is, in that species, a simple recessive, while in other New World species it is not known to occur. It has been identified with the "wrinkled-leaf" mutant which is known in Egypt in varieties closely allied to Sea Island. Dr Harland has crossed crinkled-dwarf mutants in Sea Island cottons with two other New World cottons of the Upland and Peruvian groups respectively. Substantially the same results were obtained in each case. The F_1 plants were slightly modified by the mutant, showing even at this stage some incompleteness of dominance. In the F_2 formed by self-fertilisation every degree of dominance seems to have appeared in a quite unclassifiable series. It would appear therefore that the Sea Island cottons, among which this mutant occurs, differ from other New World species in a number of modifying factors, which function together to render it dominant to the mutant. Dominance in this case must have been evolved since the separation of Sea Island from the other New World cottons. The case is of special interest in opening up the possibility of examining the genetical behaviour of the actual modifiers by which dominance has been produced.

VI. FACTORS DISTINGUISHING DOMESTIC RACES.

The genetic analysis of the factors distinguishing the different races of animals and plants which have arisen under domestication is at present in a very imperfect condition. The influence of man on these species has been, broadly speaking, of two kinds; he has enhanced for his own use certain qualities, such as fleetness, fecundity, milk yield, docility and so on, which constitute the utilitarian characteristics of our domesticated species. He has on the other hand persistently favoured novelties of all kinds, oddities in form and coloration, or in movement, as in the tumbler pigeon or "waltzing" mice. It is with this latter class of variations, almost exclusively, that genetic analysis has been successful, and there can be but little doubt that we are dealing here with the results of mutations similar to those which have been known to occur as such, and which had been, in all probability, occurring occasionally in the wild population for ages prior to domestication. It is not surprising, therefore, that the aggregate of available genetic results in this field should add little that is new, and should, broadly speaking, merely confirm the results obtained from the study of known mutations. That portion of the inherited variability from which new knowledge is to be expected, and which is from all points of view of the greater value to mankind, is apparently beyond the reach of Mendelian research as currently practised, and must await the development of more efficient and comprehensive quantitative methods.

In almost all species in which the peculiarities of domesticated forms have been investigated, a number of recessives, and but very few dominants, have been found. Of these dominants the great majority either show incomplete dominance or are in reality visible lethals. A single exception occurs in mice where, while the "dominant pied" and the "yellow" genes are lethals, yet the "white-bellied agouti" is a viable and apparently complete dominant to the wild, dark-bellied form. The white-bellied agouti is, however, scarcely a domesticated race, since it has been repeatedly caught wild, and we should perhaps more properly regard the wild mouse as possessing two forms, suited perhaps to different ecological situations, but distinguished only by a unifactorial difference. In rabbits, too, the dominant black, and the dominant, or English, white, are incompletely dominant, but one exceptional case occurs to the rule ordinarily governing allelomorphic series, in that the Himalayan breed seems to be completely dominant to the albino, with which it is allelomorphic. In guinea-pigs the reversed or rosetted fur is dominant to the wild form, but in this case there is no doubt that the condition has been much enhanced by human selection, for the basic gene for reversed fur, when alone, seems only to affect a small area in the hind leg. Whether this basic gene is really a dominant when introduced into the wild cavy has, I believe, not yet been determined.

In contrast to such rather equivocal exceptions as have been mentioned, the domestic fowl supplies in its various breeds a large number of non-lethal genes which are dominant to those of the presumed ancestor, *Gallus gallus*. These include a dominant white and a dominant black affecting the plumage, the sex-linked dominant "barred" which introduces a white bar or bars on the feathers, and a factor which replaces the black breast of the wild cock by buff or chestnut, a factor for black internal pigment, and among structural characters factors for polydactyly, feathered feet, and crest, and for the comb characters rose, pea, and duplex. The evidence for dominance is in many of these cases somewhat vague, being usually derived from crosses between breeds in which many other factors are involved, and several of them, for example both pea and duplex combs, are recorded as showing very variable degrees of dominance in different breeds. Moreover, in some cases, such as the sex-linked gene for silver, which are generally spoken of as dominants, the term has evidently been used extremely loosely, since the heterozygote is usually, if not invariably, of quite intermediate appearance. Nevertheless, looking at the mutant genes in domesticated poultry in the aggregate there can be no doubt that cases of regular and apparently complete dominance do occur with a frequency entirely unparalleled in any other domesticated plant or animal.

It should be noted that in poultry ordinary recessives, such as recessive white, and silky feathers, are quite frequent, as also are visible lethals; what is exceptional and what, on any view, must require a special explanation, is the high frequency of fully viable and complete, or nearly complete, dominance of the domesticated over the wild character, which is found in this species. The phenomenon is not found in other domesticated birds; it is also noteworthy that none of the factors under discussion is known to have arisen from a recorded mutant; all are characteristic of existing breeds, some of which must be extremely ancient. These facts suggest that the cause of the phenomenon may usefully be sought in the early history of the domestication of this species.

I formerly thought that the theory of the selective modification of dominance had no solution to offer of this particular problem. On considering, however, the facts which suggest that special causes may have been active during the earlier stages of domestication, I have come to see (1928 *b*) a possible explanation which seems at the moment not improbable, and which has the advantage of being susceptible of experimental verification. It is evident that many mutations, which in the wild state were kept rare by counter-selection, have been in domestication not merely sheltered from competition, but favoured by man for their novelty. The mutant forms are valued by man, and are regarded by him with interest, and in some historical cases with superstitious veneration. In most species the novelties appear as recessive segregates, and can at once be bred true. It appears to me that the exceptional circumstance needed to explain the case of *Gallus* lies in the fact that the domestic hen is, in its own country, constantly liable to be mated by the wild cocks; this is known to be frequent in India at the present day, and must have been the prevalent condition in the early stages of domestication by jungle tribes. In these circumstances the only mutant novelties which could be established in the domestic flocks would be those in which the heterozygote differed somewhat from the wild type. The selected mutations must, in fact, be not completely recessive. Moreover, the distinctions of the breed could only be maintained by human selection, and such selection would necessarily favour those individuals which differed most clearly from the wild type, or, in fact, those in which the mutation was least recessive or most dominant.

This case of human selection for dominance of the mutant differs from natural selection for dominance of the wild type in that, whenever the brood is half-wild, the whole population exposed to selection consists of heterozygotes, instead of only one in some five or ten thousand. Its evolutionary effect in the absence of inbreeding will, therefore, be correspondingly rapid, and it would not be surprising that great changes should be produced in a thousand generations, or even much less; especially if we give weight to the statements, respecting several of these mutants, that very variable degrees of dominance are shown in different breeds. It should be possible to put this explanation to an experimental proof, for, if the existing wild population has not been appreciably contaminated by inter-mixture with domesticated breeds, there is no reason to think that its reaction to these mutants should not be in its primitive condition. In this case we should expect that, if any of these mutants were introduced by continual back-crossing into a strain of genuinely wild *Gallus*, the historical process of modification would more or less rapidly be undone, and the mutant would be found to be neither dominant nor recessive, but one having a distinctly intermediate heterozygote, as in the case of the "blue" Andalusian. Even in the most favourable circumstances, this process would take several generations, and might be greatly retarded by linkage. It has, however, been possible to commence the experiment with a number of the most pronounced cases of dominance, and in four or five years it should be possible to decide whether the reaction of the wild *Gallus* to these mutants is or is not decisively different from that observed in the domesticated breeds.

VII. SELECTION AMONG MULTIPLE ALLELOMORPHS.

It has been pointed out by both Wright (1929) and Haldane (1930) that, in certain cases, the recessiveness of a mutation might be the inevitable consequence of the biochemical rôle played by a gene, or its immediate products; for, if we imagine these to act as enzymes, or, in general, as components of a series of chemical reactions proceeding at a certain rate, it may well be that this rate is controlled by components of the system other than the one under consideration, and that this one is always present in saturation, in the sense that no appreciable effect would be produced by increasing the activity of the gene in question, or by decreasing it to a small extent, although a more considerable diminution might largely retard the whole chain of reactions concerned, and so produce the visible effects of a mutation. If, moreover, the genes normally present in the wild species possessed at least double the activity necessary to produce their normal effects, then the complete inactivation of one of the pair of homologous genes would be attended by no noticeable consequences; whereas, if both were replaced by inactive allelomorphs, an appreciable change, such as the suppression of a particular pigment, might ensue. Slender as the knowledge, which we at present possess, appears to be for judging of the plausibility of such a situation, it does not seem to the writer improbable that many genes do in fact act in this way; though, since the system requires us to identify the maximal possible activity of a gene with its optimal activity, the existence of such systems would seem to require rather an evolutionary than a purely biochemical explanation. An example of such a system has been elucidated by Stern (1929), who has shown that the normal dominant condition of the mutant "bobbed" can be built up by accumulating a number of recessive allelomorphs carried in additional *Y*-chromosomes. The mutant bobbed gene is thus seen to be not the absence of its normal allelomorph, but a particle capable of exercising the same effects, though acting with considerably lower intensity.

Both Wright (1925) and Ford (1930) have noted that such systems could have been brought about by the action of selection upon modifying factors; for the particular saturation value beyond which further activity is ineffective must be determined by other components of the system, and can therefore be changed to a higher or lower value by the modification of these components. Haldane (1930) has further put forward the valuable suggestion that many different allelomorphs of the wild-type gene may exist, which, if they all act up to the saturation intensity, would in such cases be indistinguishable. When, however, any of these come to be combined with an inactivated mutant allelomorph, only those with a "factor of safety" of at least two would produce the normal effect. Consequently, in such heterozygotes the more potent members of the allelomorphic series would possess a selective advantage, and would thus come to prevail throughout the wild population. By these two processes of modification it seems not improbable that many genes may have come to play the rôle of a component in excess in the chains of chemical reactions, through which their more important effects are brought about; though it should be noted that such a process seems merely to allow certain genes to throw on to other components of the system the responsibility for regulating the speed of biochemical reactions. Haldane's suggestion, however, of selection among multiple allelomorphs seems to be especially relevant to some of the cases of polymorphic animals which will be discussed in the following sections.

VIII. STABILITY OF THE GENE RATIOS IN POLYMORPHIC SPECIES.

The consideration of the dominance phenomena exhibited by polymorphic species has led to a very great extension of the theory of the evolution of dominance, beyond its scope as originally put forward. It has, however, resulted in so many and detailed verifications of the consequences implied by that theory, in addition to setting the genetic situation in these species in a clearer light, that no account of the theory would be complete without bringing them also into discussion. It should, however, be borne in mind that the conclusions arrived at are only on firm ground in those comparatively few cases in which preliminary genetical research has already been accomplished, and that in these cases much more decisive evidence may be looked for in the future, now that the observations of critical importance can be more clearly indicated.

Some few years ago (1927) I was led to the conclusion that polymorphism in certain butterflies, where it had been shown to depend on the segregation of one or more Mendelian factors, must imply the somewhat special conditions needed to ensure the stability of the frequencies of the different genes. Such stability, although subject to many possible influences, would find its simplest explanation if the heterozygotes could be postulated to be at a selective advantage compared to both of the alternative homozygotes. In the case of *Papilio polytes*, Fryer (1913) had shown that the two recognised mimetic types of female differed from the non-mimetic male-like female by a single dominant gene, while a second dominant determined whether the female, if mimetic, should be a mimic of *P. aristolochiae* or, in the case of the double dominant, a mimic of *P. hector*. Without in the least appreciating the significance of dominance in the interpretation of the case, I was struck by the observation of Fryer that in his experiments numerous cases of sterile unions occurred, which suggested to him the possibility of the existence of "illegitimate" pairings, analogous to "illegitimate" pollination in heterostyled plants. The observation suggested the possibility that the selective advantage of the mimetic coloration was in nature counterbalanced by an inferior fertility of the homozygous dominants, so establishing that stability of the gene ratio on which the continued polymorphism must depend. The further possibility at once suggested itself that the selective advantage of a physiological factor, such as viability, or fertility, might be capable of numerical evaluation in culture, and that by observing the relative frequencies of the different forms in the wild population we should, in such cases, have a unique opportunity of evaluating the bionomic advantage in nature of one coloration over another.

A somewhat similar but equally obscure situation is revealed by Gerould's work (1923) on the dominant white observed in the female of several species of the butterflies *Colias*, which also reveals some peculiar features suggestive of a stability mechanism governing the yellow-white gene ratio. Gerould reports that great difficulties were encountered in obtaining the homozygous white types, these difficulties being evidently connected with the occurrence of a closely linked lethal factor. When pure white broods had been obtained, in a strain apparently freed from the lethal, the failure of the males to mate caused the introduction of wild males, and these were found to bring in the lethal factor. The conclusion that this particular lethal is not apparently rare in nature, although we should expect it to die out somewhat rapidly, suggests strongly that a stabilising system must be present, and that the heterozygous white female must enjoy some selective advantage over the yellow form, although in this case the mutant cannot be recognised as mimetic. The situation is, however, much obscured by the frequent occurrence of abnormal ratios.

Cases of polymorphism permanently maintained in a species by the stability of the frequency ratio of a pair of allelomorphs supply opportunities peculiarly favourable to the selective evolution of dominance, for in these cases the heterozygotes are not extremely rare, but usually constitute a perceptible percentage of the population, instead of something like one in ten thousand, as in the case of the heterozygotes of the ordinary deleterious mutations. The development of dominance by the phase having the more advantageous appearance would be expected, therefore, in the absence of special obstacles, to be particularly rapid. It is therefore highly suggestive that in both these cases we should have inferred on other grounds that the dominant form possesses a selective advantage.

Very extensive genetical experiments carried out by Nabours in the grouse locusts *Paratettix* and *Apotettix* (1925) have shown that the very highly developed polymorphism of this group is determined by a number of genes or gene complexes which, if not allelomorphic, are very closely linked in inheritance. Each species has a relatively common form which is completely recessive to all the others, but the dominant forms, if allelomorphic, show no mutual dominance, but have heterozygotes combining the characteristics of the two dominant homozygotes. As early as 1920, Haldane suggested with respect to the grouse locusts, that the close linkage and frequent apparent allelomorphism observed was due not only to the infrequency of crossing-over in their chromosomes, but to several chromosomes received from the same parent being generally transmitted in a group to the same offspring. A similar interpretation was later put forward by Demerec (1928) with respect to the large group of closely sex-linked factors found by Winge (1927) to determine the striking polymorphism in the fish *Lebistes reticulatus*. Haldane later (1930), with the support of C. D. Darlington and C. L. Huskins, suggested that such linkage between chromosomes may be accounted for by sectional translocations, and that the dominant genotypes are themselves due to the duplication of such translocated segments. Some explanation of this kind appears to the writer extremely attractive in respect of the very close and frequent linkage observed, but not by itself as sufficient to account for the dominance, for while it is extremely probable that the addition of a translocated segment to the normal germinal outfit should produce a visible effect, one would certainly expect the effect to be intensified if the additional segment had been received from both parents. In such a case the heterozygote would be of intermediate appearance and would not closely resemble either homozygote.

If on the contrary we deduce from the observed fact of dominance the inference that the dominant colour patterns enjoy a selective advantage over the recessive, then we must postulate further, in order to maintain the equilibrium in gene frequency, that the dominant homozygotes are in some way at a selective disadvantage compared to the heterozygote. These two types are indistinguishable in appearance, and the difference between them is therefore probably of a physiological nature displaying itself in differences in viability or fertility.

It is particularly fortunate that, although the great bulk of Nabours' extensive experiments were devoted to the examination of linkage, using tests in which dominant homozygotes do not occur, yet there remains a sufficient number of cases in which insects which have received one dominant from each parent have been interbred with mates of like constitution. In such cases, apart from the rare recombinations, we should expect to obtain a number of heterozygotes equal to the sum of the numbers of the two dominant homozygotes. Results from no less than forty types of mating of this kind have been given by Nabours for *Apotettix texanus*. After assigning to their proper class all recombinations capable of separate classification, we find that in the aggregate 4309 homozygotes and 4617 heterozygotes survived to be classified. This is a very considerable deficiency of homozygotes, since it exceeds three times its standard error. The viability of the homozygous dominants appears from these data to be only about 92·9 per cent. of that of the heterozygotes.

These aggregates are of course extremely composite, since many different dominants, or compounds of dominants, have entered into the tests. If these are examined separately, and the four cases for which only a few offspring are recorded are set aside, there remain fourteen different dominants, or compounds, for which from 200 to 2300 offspring have been recorded. Of these six show an individually significant departure from expectation, and in every case this departure is in the direction of a deficiency of homozygotes. Of the remaining eight cases, six show a similar deficiency, and two an excess; the departure from expectation being, in these eight cases, no more than, on the numbers recorded, should be ascribed to random fluctuations.

There is therefore a substantial experimental basis for asserting that the homozygous dominants, or a large number of them, suffer from some degree of inviability. The data presented, it must be remembered, are only a by-product of researches not especially intended to study viability; only the extent of the observations, and the completeness of publication, has made it possible to verify the theory so far. A more deliberate investigation would necessarily include a consideration of the age up to which differential viability shows itself, whether it is to any considerable extent conditioned by environmental circumstances, and whether it is accompanied by differential fertility. The data for other species, which have not so far been fully published, should also be of value. As far as the available facts go, however, it is clear that, unless countervailing influences are at work, the dominant genes are at a selective disadvantage in the homozygotes, and that selective equilibrium can only be maintained by the bionomic advantage conferred in nature by the dominant colour patterns. If it should prove that the physiological disadvantage of the homozygote can be measured in culture, it should further be possible, from the natural frequencies of the different forms, to evaluate this bionomic advantage numerically.

IX. SEX-LINKED DOMINANTS IN *LEBISTES*.

In the fish *Lebistes reticulatus* Winge (1927) has recorded a situation in some respects remarkably parallel to that found by Nabours in the grouse locusts. Here also we find the features of polymorphism, close linkage, and a series of dominant variants. In two respects, however, the cases are sharply contrasted. In the grouse locusts the polymorphism is equally displayed in both sexes, and the pattern genes, though so closely linked among themselves, are not genetically associated with sex determination. In *Lebistes* seventeen out of the eighteen variants studied are sex-linked, and the polymorphism is almost completely confined to the male. Since in this fish the male is heterogametic, it would be impossible to study dominance in the sex-linked factors, but for the circumstance that crossing over occurs between the *X* and the *Y*-chromosome. Certain genes therefore can be introduced into the male either in the *X*, or in the *Y*-chromosome, or in both. The coloration produced being the same in all three cases, those genes which have been tested are properly classed as dominants. With respect to the autosomal gene (*zebrinus*) this also is dominant in the male, though in the female it should be classed as recessive, for in certain homozygous females the barring has manifested itself; otherwise, save in certain intersexes, the pattern genes produce no effect upon the female.

Dr Winge has informed me of the very important conclusion that whereas in the cultivated races one or more dominants are commonly found in the *X*-chromosome, yet in wild specimens this chromosome is usually "empty," that is, recessive in respect of all factors. This observation seems to place beyond doubt the conclusion, which could only have been guessed from the incidence of dominance, that in nature the variant genes are advantageous in the male but deleterious in the female fish. For, since germinal interchange undoubtedly takes place between the *X* and *Y*-chromosomes at a small but measurable rate, the difference of gene ratio observed in nature can only be maintained by the constant elimination of dominant genes in the *X*-chromosome, and their constant multiplication in the *Y*-chromosome. On this view, taking into account the fact that the gene ratios established in nature must in fact be stable, the theory of the evolution of dominance by selective agencies leads to consequences in very complete accord with the facts ascertained by genetical research. First, we should expect the variant forms to become dominant in the male, and recessive in the female fish; next, that continued counter-selection in the female should obliterate entirely, in this sex, the effects of those genes which were capable of crossing into the *X*-chromosome, and there giving rise to occasional homozygous females. The close sex-linkage of these genes is also a natural consequence of the same situation, for favourable selection in the *Y*-chromosome with counter-selection in the *X* must constantly favour those genotypes in which linkage with the sex-determining portion of the *Y*-chromosome is closest. Such selection may thus have built up the system of close sex-linkage which is now found. Moreover, close linkage with sex may have enabled certain variants, beneficial in the male, to have established genetic stability, for, had they been autosomal, their deleterious effect in the female might have definitely outweighed their genetic advantages, and thus have prevented them from contributing to the natural polymorphism. It is moreover striking, though it is perhaps a coincidence, that the one variant whose effect has not been entirely suppressed in the female is the only one that still stands outside the sex-linked system. Apart, however, from this fact, which might be interpreted as indicating that *zebrinus* is the latest addition to the collection of mutant genes by which polymorphism is determined, it is clear that the hypothesis of dominance modification enables us to interpret the remarkable genetic situation in this species as flowing from a few relatively simple causes; and it is difficult to imagine how the observed facts could more closely simulate those to be anticipated from the theory. The male in culture swims in constant attendance upon the female; and it is natural to interpret the brilliant spots and markings produced by the dominant genes as epigamic.

X. THE ASSOCIATION OF POLYMORPHISM, CLOSE LINKAGE AND DOMINANT VARIANTS.

It is sufficiently remarkable that the two cases of polymorphism hitherto considered, in the grouse locusts and the fishes, belonging to different phyla of the animal kingdom, should resemble each other in three such striking and distinct peculiarities as polymorphism, close genetic linkage, and the contrast among the forms occurring in nature between a single recessive form and a large number of dominant variants. That we are here dealing with a causal connection is shown by the genetic behaviour of the polymorphic land snails *Helix hortensis* and *nemoralis*. Little has so far been published on the genetic work which has been done in these species, though I understand that a memoir by Captain Diver is shortly to be expected. It is, however, known that the greater number of the commoner variants affecting the ground colour of the shell, the suppression of one or more bands, the confluence of the bands, or their discontinuity, are produced by factors dominant to the standard or typical form, which, on a yellow background, has five distinct and continuous bands. Further, it is known that these factors are either allelomorphic or so closely linked in inheritance that recombinations have seldom if ever been observed in culture. The parallelism with the grouse locusts is thus extraordinarily complete, although there are among these snails some variants, such as partial albinism and dilute coloration of the bands, which behave as recessives. In the case of the snails too we have what is lacking in most other animals, fossil evidence that polymorphism of the same kind, and with approximately the same frequency ratios, has been present from a very early period (Diver, 1929). A stability mechanism controlling the gene ratios may therefore be postulated with some confidence, and, on the analogy of the grouse locusts, we should expect to find that these dominant variants produce colour patterns which are in some respects more advantageous in wild conditions than the typical pattern, but that this advantage is counterbalanced by an inferior viability or fertility of the homozygous dominants. The fact that Mendelian theory provides the numbers to be expected in broods of mixed composition, on the assumption that viability is equal, should make it possible, as with the grouse locusts, to compare their viability under very closely controlled conditions; and with the assistance of the Oxford Evolution Fund I hope to breed sufficient numbers to ascertain whether, in viability also, the snails will parallel the facts observed in the grouse locusts.

It is obvious that in the case of these polymorphic species, occurring in widely separated branches of the animal kingdom, the phenomenon of dominance has only provided the first clue towards unravelling the complexities of their genetic and evolutionary situation. In the case of *Lebistes* there are, as we have seen, some grounds for regarding the close sex linkage as either the consequence, or the condition, of the balance of selective forces acting in different directions in the male and the female. On the other hand, the colour patterns in the grouse locusts are not sex limited, nor sex-linked in inheritance, and the snails, being hermaphrodite, could not show either effect. What, then, is the meaning of the extremely close linkage within or perhaps between chromosomes observed in these two cases?

It is, strictly speaking, beyond our immediate subject to speculate on this question. Yet a consideration of the obstacles which extremely close genetic linkage must oppose to the normal evolutionary development of a species, does seem to supply a rational explanation of the method of obtaining improved colour patterns, by means of partially inviable mutants, which seems to have been adopted in the grouse locusts, and possibly in the polymorphic snails. Any considerable change in the evolution of a species from its ancestral form at a remote geological period must have involved numerous genetic substitutions. The genetic novelties ultimately adopted must, as far as we know, have originated in mutations, and have won their way gradually from extreme rarity to an ultimate predominance or universal prevalence in the loci in which these genes are situated. Where free recombination is possible, hundreds of such improvements may be in progress simultaneously, the greater improvements gaining ground more rapidly, but in no way impeding the progress of such slighter improvements as may at the same time be taking place. If, on the contrary, recombination were entirely suppressed, then all the possible genotypes in the species will compete with one another like a system of multiple allelomorphs, and any mutation providing only a slight advantage to the species, in survival and reproduction, will be threatened throughout the long period which it requires to spread through the species, by the danger of a more advantageous mutation occurring elsewhere, which will inevitably thrust it aside. The smaller the advantage conferred by a mutation the greater is this danger, both because more advantageous mutations are more frequent, and because the time will be longer during which they must fail to occur, if our mutation is to be successful. In such a species therefore genetic improvements must take place in succession, one at a time, the weaker always making way for the stronger.

Such a situation would be normal in organisms without sexual reproduction. It is a more extreme condition, probably, than is found in any sexually reproducing form. Nevertheless, in a lower degree it must be approached by any organism whose germplasm is tied up into one or a few closely linked complexes; and in such organisms we may reasonably infer that the normal evolutionary process is not available for relatively slight or unessential improvements, being wholly occupied with matters of greater importance. Now the system of obtaining improved colour patterns, such as appears to fit the facts with the grouse locusts, by dominant mutations, possibly duplications, which are deleterious in the homozygous phase, raises the problem of why the recessive cannot itself be modified to a more advantageous pattern, and so supersede the dominants, with which it appears to be now in equilibrium. The possibility at once suggests itself that the colour pattern is not among the more important matters with which its evolution is urgently occupied. Possibly its sense organs, or its digestive system, or its reproductive instincts, are of more real importance to the insect; at all events we have no reason to suppose that a species with such close linkage as the grouse locusts is in a position to seize upon such a trifling advantage as an improved colour pattern might confer.

It is this point of view which brings out one of the most attractive features of Haldane's theory that the dominants are due to the duplication of a chromosome, or fragment of chromosome; for such a fragment supplies a tract of the germplasm, mutations in which are judged solely by their success in the particular dominant in which they occur. Such a tract may be regarded as set apart especially for the improvement of a particular heterozygote, or in less degree of the corresponding homozygote. Consequently, though they may compete among themselves, mutations in this tract are shielded from the competition of the mutations of higher selective value, occurring in that part of the germplasm, which is common to the dominants and the recessive alike. The modification of dominance would on this view take place by a process closely analogous to the selection of multiple allelomorphs suggested in another connection by Haldane. The exceptional conditions induced by close linkage, by the obstacle which it opposes to normal evolution by gene substitution, makes it possible for abnormalities, such as duplications, occasionally to possess a selective advantage. If, as is extremely probable, they are injurious when homozygous, they will set up the stability of the gene ratio needed for polymorphism.

When the advantage lies in the external appearance, the polymorphism will be manifest, and the variant form will tend to become dominant.

XI. SUMMARY AND CONCLUSION.

We can now attempt to draw together the several groups of observational facts upon which the theory of the evolutionary modification of dominance is based, and upon which it finds a simple and coherent explanation. The theory itself is the simple outcome of the view which, with increasing knowledge, has impressed itself more and more upon geneticists, that the effects of Mendelian factors are largely susceptible of modification through interaction with other factors in the germinal complex; it applies this generalisation particularly to the modification of the heterozygote, which, since it contains both of two alternative genes, might be expected to be particularly susceptible of modification, in those respects in which these genes produce different reactions. By its aid we can appreciate why the deleterious mutations commonly occurring in wild species, and the fancy novelties favoured by man in his domesticated animals and plants, should generally be recessive, while at the same time the variant forms of species polymorphic in nature should generally be dominant. We have seen that the special group of dominants found in domestic poultry may be interpreted without assuming for *Gallus* a special and progressive evolutionary tendency, unknown in other birds. In more detail, we can see why the observed absence or incompleteness of dominance is to be expected in the case of different mutants of the same wild gene, or again in the case of minute internal effects produced by mutations, whose principal visible effects are quite recessive. The theory is strongly corroborated by the numerous cases of mutants which normally are completely recessive, but which, in special genetic combinations, or under special treatments, unknown in nature, give heterozygotes distinguishable from the non-mutant homozygotes.

In many cases in which the facts so far known are extremely suggestive, further investigation should produce more decisive evidence. This is true of the poultry, in which the inference that the dominant characters of our domestic breeds will be found in the wild *Gallus* to be incompletely dominant awaits the experimental test. It is true also of the very important case in cotton, where it has been possible to introduce a recessive mutant found in one species into other related species, in which it does not naturally occur, and in which the evidence so far available shows it to be incompletely recessive. The theory has been verified by the imperfect viability of the homozygous dominants in the grouse locusts, but still remains to be verified in the case of the polymorphic land snails. In both these cases, however, more extensive observations, in conjunction with the enumeration of the forms found in nature, are needed to put upon a quantitative basis the inference of a bionomic advantage of the dominant phenotypes. Even in the case of *Lebistes*, where, owing to the sexual differentiation, the consequences of the theory have been verified in the greatest detail, genetic tests on a sample of the wild population, on a scale sufficient to ascertain the frequency of colour genes in the *X*-chromosome, are needed to put the selective situation beyond a doubt. It is obviously also of great importance that other cases, where polymorphism is less pronounced, should be investigated, with particular attention to such physiological factors as may affect the fertility or viability of the different genotypes. These have hitherto appeared rather as an obstacle to genetical research, than as a primary object of study; although, in respect of juvenile inviability, the genetic method offers particularly favourable conditions for its measurement.

An interesting feature of the whole subject is that in nearly every case we are concerned with a minor or secondary by-product of selective action. Anyone who accepts the view, which was propounded by some of the earlier geneticists, that selective agencies have been ineffective or unimportant in the morphological evolution of living forms, must of necessity, irrespective of the evidence, reject the view that it has been influential in the present group of cases. When, however, the matter is viewed, not with dogmatic partisanship, but in relation to the calculable magnitudes of the selective agencies at work, and to the known effects of selection in artificial cultures, it is clear, as we have seen, even with the extremely minute selections favouring recessiveness in the mutants of *Drosophila*, that they are quantitatively of a magnitude sufficient to have produced the effects ascribed to them, provided their action has not been obstructed or opposed by some unknown and hypothetical cause. It is certainly astonishing to consider that the heterozygotes of, perhaps, some thousands of lethal mutations in *Drosophila* have each been modified back to normality, yet this is scarcely more astonishing than the admitted fact, that each of these genotypes does, on a different biochemical foundation, succeed in building something so nearly resembling the normal fly as to be indistinguishable from it; and a theory, which exhibits so remarkable a fact as this as a natural consequence of the action of known causes, cannot properly be debited with the astonishment which such a fact admittedly produces.

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THE EFFICIENCY OF THE CORRELATION COEFFICIENT FOR ESTIMATING LINKAGE INTENSITIES

A SEARCH for a rapid and reliable method for estimating linkage intensities led Y. Takezaki (6) in 1925 to propose a formula based on the method of treating the fourfold table of phenotypic frequencies as a correlation table. Working quite independently, F. V. Owen (5) developed the same method in 1928. Both authors presented tables to facilitate the rapid calculation of linkages by this method.

In the F_2 generation of a cross between parents with two factor pairs differing there are to be expected, normally, four classes of zygotes. These may be designated AB, Ab, aB and ab. If the number of individuals obtained in each of these four classes is designated as a, b, c and d, respectively, and the total number observed as n, the value of r^2 (derived originally for the fourfold table by Boas (1), Johannsen (4) and Yule (7) is given by

$$r^2 = \frac{(ad - bc)^2}{(a+b)\ (c+d)\ (a+c)\ (b+d)} \qquad \text{I}$$

For a, b, c and d we may now substitute the values of their expectations in terms of the proportion, p, of the AB and ab gametes, namely

$$\frac{n}{4}(2 + p^2, 1 - p^2, 1 - p^2, p^2)$$

We then have

$$r^2 = \frac{(4p^2 - 1)^2}{9} \qquad \text{II}$$

From whence

$$p = \tfrac{1}{2} \sqrt{3r + 1} \qquad \text{III}$$

if r is taken to be positive when ad exceeds bc. This is the formula derived by Takezaki (6) and Owen (5).

The cross-over percentage, expressed as a decimal fraction, will then be given directly by p when crosses are made in the repulsion phase and by $1 - p$ when made in the coupling phase.

Takezaki derived a formula for the standard error of his estimate of p from the assumption that the standard error of r, obtained from the fourfold table by equation II, could be equated to the standard error of a correlation coefficient derived from a normal frequency surface having the same number of observations. This mistaken assumption has led to the precision of this method of estimating linkages being greatly overestimated. On Takezaki's assumption the standard error of his estimate of p may be calculated as follows:

The variance of r (V_r) from a product moment correlation coefficient is $\frac{(1 - r^2)^2}{n}$

$$V(p^2) = \frac{9}{16} V(r)$$

Then

$$V(p^2) = \frac{9}{16} \frac{(1 - r^2)^2}{n}$$

To obtain the variance of p we divide the variance of p^2 by $4p^2$

or
$$V(p) = \frac{9(1 - r^2)^2}{64\, p^4 n}$$

And the standard error of p is

$$\frac{3(1 - r^2)}{8p\sqrt{n}} \qquad \text{IV}$$

Fisher (3, p. 249) has given a general method for calculating the sampling variance (standard deviation squared) and thence the standard error of any estimate expressible explicitly in terms of the frequencies. The method involves the differential coefficients of the function in question with respect to each observed frequency and to the total, n. Applying this method to the problem of deriving the true standard error of p we proceed as follows:

$$V(r) = V\left\{\frac{ad - bc}{\sqrt{(a+b)\ (a+c)\ (b+d)\ (c+d)}}\right\}$$

Substituting in the general equation (3)

$$\frac{1}{n}V(r) = S\left\{p\left(\frac{\partial r}{\partial a}\right)^2\right\} - \left(\frac{\partial r}{\partial n}\right)^2 \qquad \text{V}$$

where r is the value calculated from the fourfold table and p is here, for each class of zygotes, the probability of an F_2 individual falling in that class. Differentiating, we obtain

$$\frac{\partial r}{\partial a} = \frac{d}{\sqrt{(a+b)\ (a+c)\ (b+d)\ (c+d)}} - \left\{\frac{ad - bc}{(a+b)\ (a+c)\ (b+d)\ (c+d)}\ \frac{(a+b+a+c)\ (b+d)\ (c+d)}{2\sqrt{(a+b)\ (a+c)\ (b+d)\ (c+d)}}\right\}$$

$$= \frac{d}{\sqrt{(a+b)\ (a+c)\ (b+d)\ (c+d)}} - \tfrac{1}{2}r\left(\frac{1}{a+b} + \frac{1}{a+c}\right) \qquad \text{VI}$$

$$\frac{\partial r}{\partial b} = \frac{-c}{\sqrt{(a+b)\ (a+c)\ (b+d)\ (c+d)}} - \tfrac{1}{2}r\left(\frac{1}{a+b} + \frac{1}{b+d}\right) \qquad \text{VII}$$

$$\frac{\partial r}{\partial c} = \frac{-b}{\sqrt{(a+b)\ (a+c)\ (b+d)\ (c+d)}} - \tfrac{1}{2}r\left(\frac{1}{a+c} + \frac{1}{c+d}\right) \qquad \text{VIII}$$

$$\frac{\partial r}{\partial d} = \frac{a}{\sqrt{(a+b)\ (a+c)\ (b+d)\ (c+d)}} - \tfrac{1}{2}r\left(\frac{1}{b+d} + \frac{1}{c+d}\right) \qquad \text{IX}$$

Since the expected frequencies in the four classes are equal to

$$n\left\{\frac{2+\theta}{4}, \frac{1-\theta}{4}, \frac{1-\theta}{4} \text{ and } \frac{\theta}{4}\right\},$$

where $\theta = p^2$, we now substitute these values for a, b, c and d in equations VI, VII, VIII and IX, respectively, and obtain

$$\frac{4\theta}{3n} - \frac{2}{n}\left(\frac{4\theta - 1}{3}\right)\frac{2}{3} = \frac{4\ (1-\theta)}{9n} \qquad \text{X}$$

$$-\frac{4\ (1-\theta)}{3n} - \frac{2}{n}\left(\frac{4\theta - 1}{3}\right)\frac{4}{3} = -\frac{4\ (1+5\theta)}{9n} \qquad \text{XI}$$

$$-\frac{4\ (1-\theta)}{3n} - \frac{2}{n}\left(\frac{4\theta - 1}{3}\right)\frac{4}{3} = -\frac{4\ (1+5\theta)}{9n} \qquad \text{XII}$$

$$\frac{4\ (2+\theta)}{3n} - \frac{2}{n}\left(\frac{4\theta - 1}{3}\right)2 = \frac{4\ (9-9\theta)}{9n} \qquad \text{XIII}$$

Since n does not appear explicitly $\frac{\partial r}{\partial n} = 0$. Substituting the values in equations X to XIII in equation V, squaring and multiplying by the expected frequencies

$$\frac{1}{n}\,V(r) = \left(\frac{4}{9n}\right)\,\frac{1}{4}\left\{(1-\theta)^2\,(2+\theta) + 2\,(1-\theta)(1+5\theta)^2 + 81\theta\,(1-\theta)^2\right\}$$

or

$$V(r) = \frac{16}{81n}(1-\theta)\;(1+25\theta-8\theta^2)$$

Since

$$V(\theta) = \frac{9}{16}V(r)$$

$$V(\theta) = \frac{(1-\theta)\;(1+25\theta-8\theta^2)}{9n}$$

The variance of $\sqrt{\theta}$ or p, will then be

$$\frac{V(\theta)}{4\theta} \text{ or } \frac{(1-\theta)\;(1+25\theta-8\theta^2)}{36n\theta} \qquad \text{XIV}$$

and the standard error of p may then be expressed conveniently as

$$\sqrt{\frac{(1-p^2)\;(1+25p^2-8p^4)}{36np^4}} \qquad \text{XV}$$

Fisher (2) has also shown that the method of maximum likelihood, in the theory of large samples, will in all cases give a standard error as small as possible. The efficiency of the correlation method can then be tested by dividing the variance for the maximum likelihood method (3, p. 250) by that of the correlation method. This quantity,

$$\frac{2\theta\,(1-\theta)\;(2+\theta)}{(1+2\theta)n} \div \frac{(1-\theta)\;(1+25\theta-8\theta^2)}{9n} = \frac{18\theta\;(2+\theta)}{(1+2\theta)\;(1+25\theta-8\theta^2)} \qquad \text{XVI}$$

will then give a measure of the efficiency of the correlation method for all values of θ ($=p^2$). The result is shown graphically in Fig. 1, as well as the apparent efficiency given by the sampling error deduced on the assumption of a normal correlation surface as given in equation IV.

It is seen readily that the curve for the actual efficiency of the correlation method calculated from the correct formula, equation XV, does not exceed 100 per cent. for any possible values of p, from 0 to 1, in accordance with the general theory. The correlation method is fairly efficient in the coupling phase and for loose linkage in repulsion. For close linkage in repulsion it is not efficient. Since there are other formulae (3), such as the maximum likelihood method and the product ratio method, which are efficient for all values of p, it would seem preferable to use these formulae in most cases.

The error formula based on the incorrect method of treating the fourfold table of phenotypic frequencies as a normal frequency surface gives more than 100 per cent. efficiency in the coupling phase, which is obviously impossible. It is only for the value of p = .50 that this formula is correct.

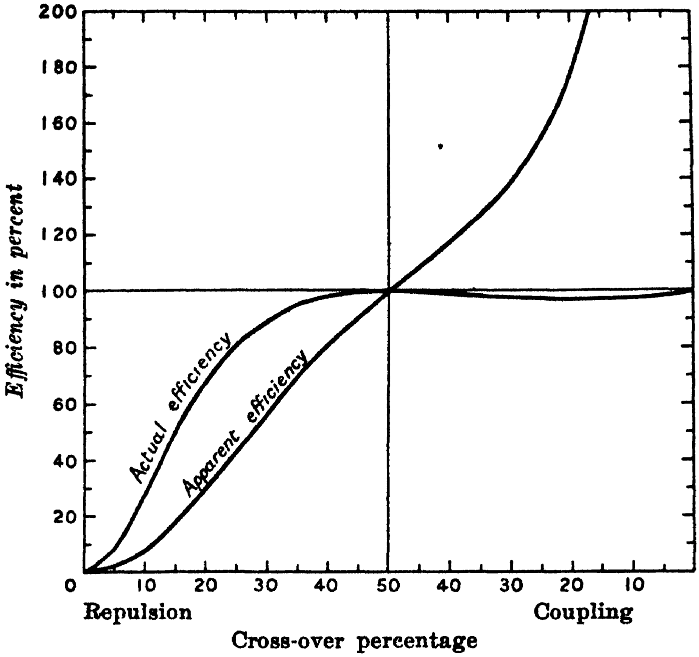

Fig. 1

Graph showing actual efficiency of correlation method for estimating linkage intensities and its apparent efficiency when the standard error of the correlation is incorrectly calculated.

For close linkage in coupling the two recombination classes, b and c, may be very small. In fact, recombinations in b or c may be entirely lacking. Under such conditions the theory of large samples breaks down and some of the efficient statistics fail. For close linkage b and c will be small numbers, either of which may be zero, while a and d will be approximately ¾ and ¼ of the sample respectively.

If b and c are small compared with a and d, then r may be expressed as

$$\left(1-\frac{bc}{ad}\right)\left(1-\tfrac{1}{2}\frac{b}{a}+\quad\right)\left(1-\tfrac{1}{2}\frac{c}{a}+\ldots\right)\left(1-\tfrac{1}{2}\frac{c}{d}+\ .\ \right)\left(1-\tfrac{1}{2}\frac{b}{d}+\quad\right)$$
$$=1-\tfrac{1}{2}(b+c)\left(\frac{1}{a}+\frac{1}{d}\right),$$

neglecting squares and products of $\frac{b}{a}$, etc.

Then

$$p=\tfrac{1}{2}\sqrt{4-3/2\,(b+c)\left(\frac{1}{a}+\frac{1}{d}\right)}$$
$$=1-3/16\,(b+c)\left(\frac{1}{a}+\frac{1}{d}\right)$$

to the same approximation. Putting the limiting values $a = \frac{3}{4}n$ and $d = \frac{1}{4}n$ in the expression, $p = 1 - \frac{b + c}{n}$. This is the same result as is given for this case by Emerson's method and by maximum likelihood.

Contrast the product method

$$\frac{(1 - p^2)^2}{p^2 (2 + p^2)} = \frac{bc}{ad}$$

When b is 0 and c is not, cross-overs must have occurred, and yet $1 - p$ is estimated to be exactly zero, which is manifestly wrong. It would seem, therefore, that the product ratio method should not be used when the observed numbers in the b and c classes are very small, *i.e.,* less than a total of about ten. For very close linkages in coupling when b and c are small the maximum likelihood, Emerson's or the correlation method would be preferable to the product ratio. The maximum likelihood method might claim a theoretical advantage since it is efficient for all values of p.

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THE GENETICAL INTERPRETATION OF STATISTICS OF THE THIRD DEGREE IN THE STUDY OF QUANTITATIVE INHERITANCE

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For the past 20 years genetical methods have gradually been made more and more familiar to the practical breeders of plants and animals, upon whom the improvement for human use of the domesticated animals and cultivated plants finally depends. During this period it has become increasingly clear that the hereditary mechanism is well represented by the Mendelian scheme, as extended mainly by the work of the Drosophilists. It has been equally clear, however, that in all the practical problems of animal or plant improvement we are invariably faced with quantitative characters, which have shown themselves to be entirely intractable by the familiar genetical methods. These methods rest primarily upon the recognition of the effects of different single factors, and when these can be recognized the study of their effects in combination follows as a matter of routine. When individual factors cannot be recognized the analytic method of genetic study cannot even be commenced, and the question arises as to whether genetics as a science has any further resource to offer.

The successes of analytic genetics have been obtained mainly with the numerous deleterious recessives which are abundant in most species, with certain easily recognizable characters of practical importance to the plant or animal breeder and with fancy characters such as the crest, or silky plumage in the fowl, which, however attractive to fanciers, cannot be regarded as of general utility to mankind. The development of the quantitative characters on which practical utility is founded owes very little to genetic analysis except in so far as it has been demonstrated that it depends on a definite gene complex. This is implicit in the study of the individual effects of Mendelian factors without the means of evaluating the mass effects of a large number of minor factors severally influencing the utility character. We would stress, however, that the study of the metrical characters is not only of utilitarian interest. The nature of the heritable elements which cause continuous or fluctuating variability must indeed be studied if progress in this direction is to be made possible; but such studies are also essential for an understanding of the evolutionary process by which organisms have been brought to their present state of organization and adaptation.

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Most of the many workers who have attacked the problem of quantitative characters have carried their work far enough to verify that the heritable variability available was probably due to a large number of Mendelian factors, interacting generally in a cumulative manner. The principal criterion has been the greater variance of the F_2 as compared to the F_1 sample. These samples are, however, generally of very different magnitude, and care has not always been taken that the conditions of culture have been such as to make the comparison a valid one. It would seem essential for the purpose that the non-genetic causes of variability should be carefully equalized. With plants, the cultures to be compared should be grown in the same year, and if, as is probable, the F_2 is the larger culture, its variability should be estimated only within areas of the same size as that occupied by the F_1; for, with uniform seed, we can always obtain a higher variability by using a larger area.

In the case of this comparison of variance, a biometrical technique has been used to verify a genetical conclusion. In seeking for further points in the genetical situation which biometrical methods, combined with an adequate cultural technique, might be able to evaluate, it should be borne in mind that, as a school, the biometricians have shown themselves singularly unreceptive to genetical ideas. Methods which are genetically appropriate will not therefore be found ready made, and constants such as the correlation coefficient, which have been introduced with the highest biometrical testimonials, while they probably have, in suitable cases, an appropriate use, have assuredly done as much to confuse as they have to clarify the subject.

In studying the properties of a system of interacting factors it has been shown (FISHER 1918) that departures from the simple additive law of interaction will usually have effects somewhat similiar to non-heritable modifications. We may therefore be confident that, even if a strictly additive interaction is not exactly realized, the mass effects of segregation in a large number of factors will closely simulate those of simple cumulative systems. In such a system certain special quantities, of which the mean and the variance are examples, possess the remarkable property that each is simply compounded of contributions derived from the several factors acting singly. Thus the heritable variance observable among any group of organisms may be regarded as the sum of the variances due to the individual factors. The portion of the variance which is heritable may be easily estimated from the covariances or mean products of the measurements of related individuals so that, without being able to recognize any single factor, we have a direct means of estimating their total contribution to the heritable variance.

This, however, is not enough to evaluate the selective potentialities of the population under examination. A number of further questions present themselves, most of which must at present remain unanswered. The same total variance might be contributed by a few factors each having a relatively large effect or by a multitude of smaller modifiers. In both cases progress can be made at once by selection, but whereas in the first case such progress will soon be accompanied by a decrease in the variance available, and will therefore soon be slowed down, in the second case progress can be continued much further in the same direction without the introduction of fresh material. Equally important, and fortunately less elusive, is the incidence of dominance; for mass selection will be far more successful in establishing recessives having a desirable effect than in establishing dominants of like effect, and this provides an obvious reason why, in material which has already, consciously or unconsciously, been much selected, the recessives are generally found to be variants in the direction which is judged to be disadvantageous.

We shall give some examples of the kind of data in which this bias in the prevalent direction of dominance, which selection must tend to introduce, appears to be shown. For the moment let us notice that effective biometrical methods of evaluating this bias will be of immediate practical value in the evaluation of the selective potentialities of a given population. From the purely scientific point of view it is also of importance that the dominance bias constitutes an existing record of the prevalent direction in which selection has acted in the immediate past. A geologist, by examining the population of individuals existing at a given horizon, might be able not only to specify the mean value and the variance of any measurement in this population, but might have a direct indication of the direction in which this measurement was in process of change.

STATISTICS OF THE THIRD DEGREE

In the study of the various methods by which the effects of biassed dominance may be brought to light, we shall be invariably led to the use of statistics of the third degree. Our knowledge of these quantities on the algebraic side is at present very incomplete. The birth of modern statistics during the past generation may be typified by the transfer of attention from the totals and means characteristic of simple accountancy to the statistics of the second degree, the sums of squares and products, on which the whole apparatus of the calculus of correlations, or in more recent times of the analysis of variance and covariance, has been built. Statistics of the third and higher degrees have, of course, been used in fitting frequency curves and surfaces, but merely to evaluate empirical and arbitrarily chosen mathematical constants; and their practical inappropriateness for this purpose has been shown by their low efficiency in, for example, fitting the parameters of the Pearsonian curves. There is at present no comprehensive method of handling the statistics of the third degree analogous to the analysis of variance and covariance, to which nearly all work with second degree statistics can be reduced. Consequently, the methods we shall illustrate will probably be found to be capable of much improvement, and no exactitude can be claimed for the estimates of sampling error, or in consequence for the tests of significance. This, however, is a drawback which we may expect to be remedied with equal pace with the improvement of the experimental data, to which these methods may be applied.

In the case of statistics of the second degree we distinguish between the variance derived from the squares of the values of a single variate, and the covariance derived from the products of the values of the two different variates. The corresponding statistics of the third degree are of three kinds: (a) those derived from the cubes of the values of a single variate, (b) those derived from the product of one variate with the square of a second, and (c) those derived from the product of three different variates. It will be seen that all three types are, in different cases, of value. For each type we must throw our calculations in such a form as to obtain an unbiassed estimate of some parameter which, like the variance, satisfies the cumulative property, and which in consequence is interpretable in terms of the individual factors of the Mendelizing system.

For example, from a series of values of a single variate we can calculate the three statistics of the first, second and third degrees, namely,

$$k_1 = \frac{1}{n}S(x)$$

$$k_2 = \frac{1}{n-1}\left\{S(x^2) - \frac{1}{n}S^2(x)\right\}$$

$$k_3 = \frac{n}{(n-1)(n-2)}\left\{S(x^3) - \frac{3}{n}S(x^2)S(x) + \frac{2}{n^2}S^3(x)\right\}$$

where S( ) stands for summation over the sample observed, and n is the sample number, which are equivalent if $\bar{x}$ is the mean, to

$$k_1 = \bar{x}$$

$$k_2 = \frac{1}{n-1} S(x - \bar{x})^2$$

$$k_3 = \frac{n}{(n-1)(n-2)} S(x - \bar{x})^3.$$

Then it has been shown that k_1, k_2, k_3 are unbiassed estimates of quantities K_1, K_2, K_3 characteristic of the population sampled and possessing the cumulative property.

A. B. D. FORTUYN (1931, p. 163) gives eight seriations for the frequencies of different numbers of tailrings in different strains of mice, derived from *Mus musculus*, *Mus wagneri* and hybrids between these two forms. He was able to show that the variation in ring number was largely hereditary, for by selection from a common stock he obtained strains with average ring numbers, 142.6 and 216.2 respectively. Selection for high values of a variate should, when applied to a symmetrical population, generally shift the value of k_3 in the negative direction; equally, selection for low values should shift it in the positive direction. The amount of these changes will depend on the number of factors present. In an ideal case in which selection in opposite directions was applied to the F_2 from two homozygous lines, so that all pairs of allelomorphs were present initially in a 1:1 ratio, the ratio of the change in k_3 to that effected in the mean k_1 should be initially

$$\frac{-\frac{1}{2}S(d^4)}{S(d^2)}$$

where 2d is the difference between the homozygous forms in any one factor, and S stands for summation over the different factors. The rate at which the third moment is modified for a given change in the mean is evidently greater, other things being equal, the smaller the number of factors to the segregation of which the variance of F_2 is to be ascribed. As it stands it affords therefore a crude method of estimating or at least of setting a lower limit to the number of factors present. Although FORTUYN'S material was not formed as an F_2 from homozygous lines, it may be of interest to point out that the high selection line has in fact a negative k_3, though, on the number counted, not a significant value. Of the seven lines given, however, two, both with high ring numbers, do show significantly negative k_3, while a third with low ring number gives a k_3 which is significantly positive. The phenomenon to be expected thus does not seem to be beyond attainable precision. The seven values are as follows:

TABLE 1

	k_2	k_3	g	n
Mus wagneri	138.8	−66 4	−.095±.228	113
LTM	142 6	−36.2	−.072±.229	111
BWTW	150.1	+2162.1	+.581±.199	149
WTW	159.0	+467.3	+.278±.182	179
Albino *Mus musculus*	189.3	−1194.0	−.526±.122	446
WM	195.0	−1667.6	−.584±.160	230
HTM	216.2	−238.5	−.258±.143	290

The best available test for the significance of k_3 (FISHER 1928) seems to be to calculate the ratio $g=k_3\,k_2^{-3/2}$; then for sampling from a normal population the true variance of g is $6n(n-1)/(n-2)(n+1)(n+3)$ and its distribution is, for samples over 100, sufficiently near to normality for significance to be inferred from the standard errors as shown in the table. The standard error will be used throughout this paper in testing significance.

This example is illustrative only of the type of biometrical effect which is not beyond experimental precision, by which direct information may be obtained as to the distribution of the heritable variance among the genetic factors present. The interpretation of any particular body of data for which this effect was measured could evidently be carried much further by estimating also such quantities as the heritable variance and those unsymmetrical effects ascribable to dominance. These latter will indeed, in practice, almost always complicate the interpretation of any data bearing on the size and number of the heritable factors.

THE EFFECTS OF DOMINANCE BIAS ON F_3 PROGENIES

The observational facts that the cross (F_1) between two strains frequently shows greater "vigour," or growth rate, than either parental type and that inbred lines frequently show a falling off in size, which is reversible by a single cross, may be interpreted either on the view that there is among the genetic factors present a pronounced bias in dominance, in the sense that greater size is more usually dominant to less size, than *vice versa*, or, on the contrary, that the heterozygote in a single factor is frequently larger than either of the corresponding homozygotes. These two views differ considerably in their practical consequences, but the contrast may be reduced to the quantitative question of the normal position of the heterozygote in a single factor relative to the two homozygotes.

Let us suppose that two homozygotes differing in any one factor differ on the average in the metrical character under observation by a quantity 2d, so that the mean values for the two homozygotes differ from an arbitrary origin (the mid-point between the two homozygotes) by $+d$ and $-d$; we may then represent the average deviation of the heterozygote from the same origin by h. If h is generally positive, or at least generally positive for the more important factors, there will be in the system of factors considered a positive bias of dominance. The heterozygote for the whole group of factors will then exceed the mean of any two complementary homozygotes from which it might have been obtained. For any factor, if h lies between the limits $-d$ and $+d$, dominance will be partial or incomplete, if it is equal to $\pm d$ dominance will be complete, but if it exceeds $+d$ we shall have a case of superdominance. We shall consider how the biometrical data from F_3 progenies may be used to calculate whether the factors present as a whole have values of h which are positive, and if so whether there is evidence that they exceed the value d.

Although the main object of this paper is to call attention to the significance of various statistics of the third degree, yet it will be convenient here to state briefly some second degree results, which, though long known in principle, have not, we believe, been developed in a form convenient for experimental utilization. The three phases of any factor, if fully viable, may be expected in F_2 in the ratio 1:2:1. It easily follows that the contribution of such a factor to the variance in F_2 will be $\frac{1}{4}(2d^2+h^2)$, when the deviations are measured from the mean, $\frac{1}{2}h$. The total observable variance in F_2 does not, however, provide a satisfactory basis for evaluating directly the sum of these quantities, since, in all quantitative characters which are susceptible to environmental influences, a positive contribution will be made by environmental modification, and it does not appear that any experimental refinement could altogether eliminate this source of error. In the case of the covariance, on the other hand, the environmental deviations will be equally frequently positive and negative, and will only lower the precision of the result by increasing the quantities upon which the estimate of error is based. The covariance is calculated from $S(x-\bar{x})(y-\bar{y})/n-1$ where x and y are the two variates and n is the sample number. With much plant material two types of covariance may be fairly readily obtained: (1) the covariance between the F_2 parent and the mean of the F_3 progeny derived from it, the contribution of each independent factor to which is $\frac{1}{4}(2d^2+\frac{1}{2}h^2)$ and (2) the covariance of parent and progeny when the F_2 are crossed *inter se* at random, the value in this case being $\frac{1}{4}d^2$. From these two quantities we can, with precision limited only by the homozygosity of the parent stocks, determine the part of the F_2 variance which is genotypic merely by taking twice their difference. What is equally interesting, the method shows a way of separately evaluating the ratio of the mean or average of the quantities h^2 to that of the quantities d^2, and so of discriminating between the hypothesis that the system of cumulative factors is one in which dominance is generally absent or slight, as is often assumed, and the hypothesis more generally favored when hybrid vigor is manifest that dominance is as complete in the quantitative factors as it generally is in factors which can be isolated for separate study.

Statistics of the second degree can obviously not distinguish whether h is positive or negative; they cannot therefore be used to investigate the extent to which dominance is biassed. Indications of this may be obtained from the first degree statistics, the means, as when an F_1 exceeds the mean of the parental values, and we infer that h is more frequently or more largely positive than negative. The comparisons of mean values, of which the most important is the difference between F_1 and F_2 is, however, a matter of some experimental difficulty, especially when the number of F_1 seeds is small, and, though they would be valuable in conjunction with other facts, by themselves they are not capable of more than a qualitative interpretation. From a set of F_3 progenies, however, it is possible to obtain three statistics of the third degree, which have a direct relevance. These are:

(1) The mean value of the k_3 from each of the progenies, to which each factor contributes $(-3/8)d^2h$.

(2) The covariance of the k_1 and k_2 in different F_3 progenies, to which each factor contributes $+h(2d^2+h^2)/32$.

(3) The k_3 of the means of different F_3 progenies, to which each factor contributes $(-3/8)d^2h$.

In respect of availability we shall show that no very extensive data are required to estimate the first of these quantities, while a larger number of progenies than in the examples to be given, with the exception of the barley data, though not an impossibly large number, would be needed to obtain good values for the second. The third would evidently be liable to large disturbances owing to the varying fertility of the areas upon which different progenies must be grown, and is in any case liable to much larger sampling errors than is the first. The first process will therefore always give the preferable value. What is important, however, is that, by a comparison of the mean value of k_3 with the covariance of k_1 and k_2, it is possible directly to distinguish between the views that an apparent effect of heterosis is due to ordinary dominance, either complete or incomplete, favoring the larger values, in which case a homozygote may be established as vigorous as any heterozygote, and the alternative view that in many factors the heterozygote is more vigorous than either homozygote. If we multiply the covariance by 4 its value will be greater than, equal to, or less than the mean value of k_3 (with sign reversed) according as h (supposed positive) is greater than, equal to, or less than +d. With true superdominance four times the covariance should have a positive value exceeding the negative average value of k_3 within F_3 progenies, while if we are confronted only with a strong positive bias of the dominance, it should at most be equal to this value. If the system were of so simple a kind that in each factor the heterozygote was equal to the larger homozygote, we should find confirmation of the fact from the equality of $S(h^2)$ and $S(d^2)$, and should know that only by specific interactions could the average be raised above the level of the multiple heterozygote. Equally, if the covariance is less than this critical value, it is clear that the possibilities of mass selection have not been exhausted.

An example may be taken from the distribution of leaf length for 13 F_3 families of lettuce given by C. E. DURST (1930, p. 266). The mean leaf length in F_1 was greater than that of either parent, while the mean in F_2 was slightly shorter than the larger leaved parent; by this indirect comparison the mean of F_2 may be judged to be about 2.2 units less than in F_1, one unit being 1.5 cm. The numbers of individuals and the values of k_1, k_2 and k_3 obtained for the 13 F_3 progenies are shown in table 2, differences of 1.5 cm being taken as one unit, the first unit being at 10 cm.

TABLE 2

NUMBER OF INDIVIDUALS	k_1	k_2	k_3
21	4.286	3.014	1.427
8	6.000	13.143	4.572
39	7.590	1.143	−0.720
26	7.615	7.126	−2.381
17	7.647	3.742	−1.146
50	8.180	6.559	−3.122
25	8.480	10.927	−24.565
47	8.936	4.061	−2.660
13	9.539	5.102	−0.975
13	9.846	8.974	−20.700
35	9.886	8.104	−9.715
53	10.585	2.786	−5.631
11	12.000	8.200	−18.333

From these we find directly the mean value of k_3 to be -6.458 ± 2.532, a negative value, as the theory has indicated, which is statistically significant, but owing to the small number of families, not well determined numerically. For the covariance of k_1 and k_2 we have $+0.492$, which is, in accordance with the theory of cumulative factors, positive, though no statistical significance is to be attached to this fact as its standard error is as high as 2.017.

The k_3 of the means of the thirteen F_3 lines is found to be -3.476 ± 6.159, a negative value, again in agreement with the theory but not significant. The sampling variance of the mean k_3 has been obtained from the formula $\{1/(n-1)n\}\ S(k_3-\bar{k}_3)^2$, where n is the number of k_3's calculated, the variance of the k_3 of the F_3 means from $\{6n/(n-1)(n-2)\}\ k_2^3$ and the variance of the covariance of k_1 and k_2 from $(1/n-1)\{V(k_1)V(k_2)+V^2(k_1k_2)\}$ where $V(k_1)$, $V(k_2)$ stand for the estimated variance of k_1 and k_2 in different families and $V(k_1k_2)$ for their estimated covariance.

It will be observed that very different numbers of plants were obtained in the different F_3 families, and consequently the precision of the statistics derived from them must be expected to vary greatly. We may anticipate in general that the best possible theoretical estimates will be something between those obtained by giving, as above, equal weights to all families, and the corresponding values obtained by weighting each in proportion to the number of plants recorded. To ascertain whether in this case weighting would give appreciably increased precision, the values were recalculated on the latter system. The standard error of the mean k_3 was reduced from 2.532 to 2.087, and that of the covariance from 2.017 to 1.427, showing that it will be found a decided advantage in the use of such data for our present purpose if the F_3 progenies are approximately equal in number.

This advantage is emphasized equally by the circumstance that the statistics derived from the means of a limited number of plants will be affected by the k_3 of their distribution about their means. Both the covariance and the k_3 of the means require for this reason a correction algebraically equal to $-\bar{k}_3/s$ where s is the number of plants per family. With variable family numbers it will doubtless be sufficient in applying this correction to use the harmonic mean of the actual numbers, but the existence of a correction of this kind is a sufficient reason for keeping the numbers of the F_3 families as nearly constant as is conveniently possible.

The fourfold value of the covariance of k_1 and k_2 is here less than the mean k_3, indicating that h is generally less than $+d$; this is true even when the considerable correction $+.360$ is added to the crude value $+.492$. No significance can, however, in this case be attached to the comparison, considering the magnitude of the standard errors. It is of interest to note also that the k_2 of the F_2 progenies, 9.536, was almost twice that of the weighted k_2 of the F_3 progenies, that is, 5.468. The contribution of a single factor to the variance of the former would be $\frac{1}{4}(2d^2+h^2)$, and to the latter $\frac{1}{8}(2d^2+h^2)$. The comparison of the two variances thus suggests that a large proportion of the observed variance was in both cases genetic in origin.

For a critical study far more extensive data would be needed. The data given here are recognized to be inadequate except to point out how the problem may be attacked. Not only would more plants be required in F_2 and the F_3 lines but the number of F_3 lines should also be increased greatly in order that these should adequately sample the segregation in F_2. With the use of sufficiently extensive data it should be possible to reduce the standard errors to limits which would permit of exact comparisons between the different statistics and to fix the prevailing ratio of h and d within reasonable bounds. Replication, as a means of reducing the effect of soil variation, would be highly desirable.

Another example will be taken from published data on inheritance of a quantitative character in maize. EMERSON and EAST (1911, p. 77) presented data on inheritance of height of maize plants from a cross of Tom Thumb pop and Missouri dent, two open pollinated varieties. A guess mean was taken at 18 dcm, 1 dcm was taken as a unit and the following table computed from the sixteen different F_3 distributions given:

TABLE 3

NUMBER OF INDIVIDUALS	k_1	k_2	k_3
40	−7.225	2.076	0.597
114	−5.114	6.102	3.145
64	−6.016	2.524	0.106
65	−0.785	2.734	−3.155
90	2.011	4.685	−2.975
85	0.306	6.310	2.698
82	1.488	6.944	−4.716
85	0.635	6.162	−7.731
82	1.598	3.503	−0.268
95	4.432	5.567	−1.393
87	1.805	5.182	−5.226
149	3.745	3.070	−0.657
87	4.207	5.422	−2.954
93	4.774	4.764	4.943
87	5.862	2.981	−0.162
76	7.145	5.512	−0.011

The k_2 of the F_2 was 12.499 ± 1.415 and the k_3 of the F_2 was -15.694 ± 8.666. From the table we find the mean k_2 of the F_3 lines to be 4.596 ± 0.389 and the mean k_3 of the F_3 to be -1.110 ± 0.821. The latter is negative as expected for a dominance bias, but cannot be considered statistically significant with the data available. The covariance of k_1 and k_2 is found to be $+1.925 \pm 1.795$, a positive value as expected, but again not significant. Twice the value of the k_2 of the F_3 is here even less than the k_2 of the F_2. The variance of the mean k_2 of the F_3 lines was obtained from the formula $\{1/n(n-1)\}S(k_2-\bar{k}_2)^2$ where n is the number of F_3 families.

In the summary of the paper by EMERSON and EAST we find a statement relative to the "lack of skewness in the F_2 frequency distributions" for the crosses employed in studies on inheritance of height of plants. Taking the total distributions for F_2 in tables 25, 26, 27, 28 and 30 and the same for the F_2 in 1911 in table 29 we may determine statistically whether these distributions were or were not symmetrical. We find that $(k_3k_2^{-3/2})$ was -0.105 ± 0.120, -0.254 ± 0.096, 0.028 ± 0.161, 0.012 ± 0.105, -0.355 ± 0.192 and -0.161 ± 0.106 in tables 25 to 30, respectively. A negative bias is indicated in four of the six crosses tested; although in the second alone is the skewness statistically significant, yet two other negative values are suggestively large.

Early studies of heterosis emphasized the fact that if dominance of growth factors were the explanation of hybrid vigor the F_2 distribution would be skew. Such was not the case, it was argued. With a large number of growth factors the F_2 distribution would tend toward the normal, and large numbers would be needed to demonstrate a negative bias. That such a negative bias is fairly common is demonstrated by the negative k_3 found from the data examined in this paper.

The maize data were obtained from a cross of two open pollinated varieties which were undoubtedly heterozygous in many of their factors for height. The data are given only to show how the problem may be attacked. Maize offers unusual possibilities for biometrical studies on quantitative inheritance. Some of the advantages of maize will be enumerated.

Selfed lines are already available which may be considered homozygous for the greater part of their growth factors. Crosses are easily made and a large number of seeds usually obtained. It would be possible, usually, to self a sufficient number of F_2 plants to obtain seed for tests in F_3 and to leave sufficient seed of the F_2 generation for comparison of the F_2 with the F_3 progenies in the following year. Since maize is very highly cross pollinated, seed from the various F_2 plants may be planted in an isolated plot also and allowed to cross *inter se*.

Parent lines, F_1, F_2, F_3 and lines from F_2 plants crossed *inter se* can then be grown in a single year in a replicated yield trial. The value of careful replication cannot be over-emphasized for such a study as a means of eliminating soil variation. Until more information is available on this point it may be suggested that, say, 50 to 100 plants in each of 100 F_3 lines ought to give sufficient data for a critical study. These might well be grown in five replicated plots of 10 to 20 plants each.

The contribution of a single factor difference to the following statistics may be noted:

Statistics of the second degree

Variance of F_2	$\frac{1}{4}(2d^2+h^2)$
Mean variance of F_3 progenies	$\frac{1}{8}(2d^2+h^2)$
Variance of means of F_3 progenies	$\frac{1}{4}(2d^2+\frac{1}{4}h^2)$
Covariance of F_2 parental value with mean of its F_3 offspring	$\frac{1}{4}(2d^2+\frac{1}{2}h^2)$
Covariance of F_2 parental value with mean of its biparental offspring	$\frac{1}{4}d^2$
Mean variance of biparental progenies	$\frac{1}{16}(4d^2+3h^2)$
Variance of means of biparental progenies	$\frac{1}{16}(4d^2+h^2)$
Mean variance of maternal progenies	$\frac{1}{8}(3d^2+2h^2)$
Variance of means of maternal progenies	$\frac{1}{8}d^2$

The biparental progenies are those obtained by crossing two F_2 plants. The maternal progenies are taken to be the progenies of plants exposed to open pollination by sister F_2 plants. Whether the multiplicity of measures also affords a method of measuring and eliminating the effects of incomplete linkage, we have not investigated; it would seem premature to attempt this until biometrical studies with visibly classifiable factors have shown what kind of disturbance is to be looked for.

Of the above statistics the four variances of individual values will usually be sensibly increased by environmental modification; the variances of the means could be freed from this bias, if the progenies are not grown on separate areas. The two covariances should be free from bias, and the experimental arrangement could be devoted solely to diminishing their sampling errors.

Statistics of the third degree

k_3 of F_2	$-\frac{3}{4}hd^2$
Mean k_3 of F_3 progenies	$-\frac{3}{8}hd^2$
Covariance of means and variances of F_3	$(h/32)(2d^2+h^2)$
Covariance of F_2 parental value and variance of F_3	$(h/16)(2d^2+h^2)$
k_3 of means of F_3 progenies	$-\frac{3}{8}hd^2$
Mean k_3 of biparental progenies	$-\frac{3}{16}hd^2$
Covariance of means and variances of biparental progenies	$-\frac{1}{8}hd^2$
Covariance of parental values and variances of biparental progenies	$-(h/32)(2d^2-h^2)$
Covariance of biparental progeny and biparental product	$-\frac{1}{8}hd^2$
k_3 of means of biparental progenies	$-\frac{3}{16}hd^2$
Mean k_3 of maternal progenies	$-\frac{3}{8}hd^2$

Covariance of means and variances of maternal progenies	$-\frac{1}{8}hd^2$
Covariance of parental values and variances of maternal progenies	$-\frac{3}{16}hd^3$
k_3 of means of maternal progenies	0

It will be noticed that the same quantities may be obtained experimentally in many different ways. This should afford a valuable empirical test as to the consistency of the genetical interpretation and will also prove valuable in detecting and eliminating the metrical bias, to be discussed later, with which statistics of the third degree, apparently with the exception of the covariance of the biparental progeny mean with the biparental product, may be affected. For the moment we need only notice that since this bias is always of the same sign, significant values of opposite signs cannot but indicate genuine genetical effects.

Some of these comparisons will be subject to greater errors than others. The previous examples show that the mean k_3 of F_3 lines is determined with much greater precision than the k_3 of the means, or the covariance of k_1 and k_2. One of the objects to be aimed at in exploratory work of this kind must always be to ascertain which biometrical values, of those which have an important genetical interpretation, can be ascertained in practice with a useful degree of precision.

In any case when both parental values are known as well as the mean of the progeny, the effect of this negative correlation of the progeny with the parental product may be exhibited by a purely biometrical procedure, one in which the relationships of the different parent stocks are not taken into consideration. We may in fact always calculate the regression of progeny values upon the three "independent" variates, maternal value, paternal value and parental product. Such a partial regression equation was calculated from data on row number from 46 backcrosses of maize kindly supplied by E. W. LINDSTROM. From data on row number of both parents and mean row number of the progeny the partial regression of mean progeny row number was found to be given by $0.4418x + 0.5148y - 0.0364p$ where x and y are the maternal and paternal row numbers and p is the parental product. The covariance of mean of progeny on the product of parental deviations was negative as expected, but the value shown for partial regression was not significant, having in this case a standard error ±.0369. The regression of progeny on mother or father was about one-half as expected.

There are, in general, two important sources of disturbance in biometrical studies concerned with a study of quantitative inheritance. The first may be termed the dominance bias. When dominance favours the larger values the segregating generations will tend to have a negative skewness (as measured by k_3). The extent of this bias will depend solely on whether dominance is complete or incomplete. The maximum skewness would be obtained when dominance is complete. With h greater or less than +d this skewness would be reduced. It is from the properties connecting h and d in different types of distributions and matings that these quantities may be estimated, as shown previously.

Another source of bias is encountered also very frequently. This may be termed the metrical bias, since it depends on the scale of measurements used. It is a not infrequent observation that the standard error of yield of a group of plots on a field of low fertility is often higher than that of plots on a high fertility field. The yields of plots from a low fertility field vary more with slight variations in soil fertility than do plots on a field which is already producing nearer to its maximum. The k_3 of yields from a group of plots varying in fertility would, in such cases, be negatively skew.

An example of this "inherent" negative bias will be taken from measurements of height of barley plants grown in different plots, with different nitrogen fertilizers, at the ROTHAMSTED EXPERIMENTAL STATION in 1928. The heights of sixteen plants were measured in each of 24 plots, the plants being selected at random within each plot. The mean height in cm to the auricle of the last expanded leaf for all observations was 37.6175 cm. The mean k_2 for the 24 plots of 16 plants each was 67.107 ± 2.078 and the mean k_3 of the same 24 plots was -194.099 ± 61.231. The covariance of k_1 and k_2 was -14.369 ± 13.248. A negative skewness is evident, indicating an inherent negative metrical bias. The covariance is also negative, again indicating the same type of disturbance. It is not significant, however. Since a normal variety of barley was used, and barley is very highly self fertilized, this bias cannot be attributed to genetic segregation for plant height factors.

These two sources of bias, the dominance bias and the metrical bias, will tend to counteract one another in the covariance of k_1 and k_2 of the lines in genetical studies of quantitative inheritance. The extent of the disturbance due to the metrical bias will depend somewhat on which statistics are used. It will be greatest in the covariance of k_1 and k_2 and will not be as serious a source of disturbance to the mean k_3.

Extensive data on height of barley plants from a cross of a two row (*Hordeum distichum nutans*) and a so-called six row variety (*Hordeum tetrastichum*) made by TEDIN and grown at Weibullsholm, Sweden, in 1930, will be used to illustrate the type of information which may be obtained from such data. This study was made entirely on F_3 material. Two hundred and seventy F_3 lines, segregating for the factors two row versus six row were grown and individual height measurements obtained on 14,759 plants in these lines. The number of plants varied considerably in the different lines but weighting was not attempted.

The mean height of all the F_3 plants was 87.4 cm. Taking 5 cm as unit the mean k_2 of the 270 F_3 lines was 3.475 ± 0.077 and the mean k_3 -2.176 ± 0.204, a negative quantity in accordance with the theory that dominance of factors for plant height should give a negative k_3. This quantity was highly significant owing to the ample data on which it was based. Another third degree statistic was also available, that of the k_3 of the means of the F_3 lines. This was found to be -0.932 ± 0.567, a negative quantity as expected but not statistically significant. Since replication of the F_3 lines was not used this quantity would not be expected to be determined very accurately. The third statistic of the third degree calculated was the covariance of k_1 and k_2. This was found to be -0.564 ± 0.124, a negative quantity that was highly significant. If the dominance bias had been operating alone the covariance should be positive. The question arises as to whether the clearly significant negative value for the mean k_3 is to be ascribed to the co-operation of both measures of bias, or principally or entirely to the metrical factor.

For moderate bias it may be anticipated that the bias in the mean of the k_3 of the progenies will be proportional to six times the mean square of their variances, that is, to 81.8478, while the corresponding bias in the covariance of the means and variances of the families will be proportional to four times the product of the mean variance of the progenies and the variance of their means. This comes to 33.6060 or more than one-third of that found for the mean of k_3. Since the negative value found for the covariance, when corrected, as before explained, for the limited number measured in each family, is just less than a quarter of the mean value of k_3, we may conclude that the negative value of the covariance may be wholly accounted for by metrical bias without abolishing at the same time the negative values for k_3. In this case, however, it is clear that metrical bias has been a major factor in both values, and even the ample material measured would not allow us to attach significance to the residual genetic effects remaining after the metrical bias had been removed. It would, of course, be not surprising with a normally self fertilized plant, such as barley, that the pronounced bias in the dominance of genetic factors, comparable with that indicated for maize, should be absent or inconspicuous.

The average height of the 2 row, heterozygous (that is, for the 2 versus 6 row factor pair) and 6 row plants in the F_3 lines segregating for the 2 versus 6 row factor pair was found to be 88.2405, 88.0035 and 83.1190 cm, respectively. The heterozygous group of plants cannot be considered significantly different in height from the 2 row plants. Apparently, therefore, as far as height factors linked with the 2 versus 6 row factor pair are concerned, no evidence of superdominance could be found. It seemed of interest to determine next the average variance (k_2) of height of the 2 row, heterozygous and 6 row plants when these were segregates in the same F_3 lines and grown on the same areas of land. The average k_2's were 87.6675, 84.1100 and 70.9825 square cm, respectively, for these three groups. The mean k_2 of the 2 row plants was 3.5575 ± 3.2275 square cm greater than the k_2 of the heterozygotes and 16.6850 ± 3.5950 square cm greater than that of the 6 row plants. The mean k_2 of the heterozygotes was 13.1275 ± 2.6500 square cm greater than the k_2 of the 6 row plants. The first of these differences is not statistically significant but the latter two are. When, however, the standard errors ($\sqrt{k_2}$) were expressed in percent of the mean heights, values of 10.61, 10.42 and 10.14 percent were obtained for the 2 row, heterozygous and 6 row groups. Apparently when the standard errors of the heights of these three classes of segregates, grown on the same small plots of land, were expressed in percent of the mean height the coefficient of variability was practically constant for the three groups.

SUMMARY

1. A genetical interpretation is given for various second and third moment statistics which are of use in studying quantitative inheritance.

2. Published data taken from lettuce and maize, and unpublished data from barley crosses are used to illustrate how the problem may be attacked. The special needs of data adequate for this purpose are illustrated, and certain possible precautions in planning the experiments are pointed out.

3. A study of the skewness of seven distributions for strains of mice selected for high and low tailring number indicated that the theoretical negative association between the statistics k_1 and k_3 in selected strains could probably be evaluated.

4. Formulae are given by which the effect of the dominance bias in the heterozygote in relation to the measurable characters of the homozygotes in F_2 or F_3 distributions or various types of crosses may be calculated.

5. The two common sources of bias (metrical and dominance) are discussed and data from a barley cross used to illustrate the results obtained when the former is of major importance.

6. Since the combined effect of the dominance and metrical biases may be obtained experimentally in many different ways, an empirical test of

the consistency of the genetical interpretations is available, as well as an opportunity of evaluating and eliminating the metrical bias.

7. Standard errors are given for the different statistics used.

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THE EVOLUTIONARY MODIFICATION OF GENETIC PHENOMENA

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The title chosen for our discussion is "Contributions of genetics to the theory of evolution," and that these contributions are of two kinds, somewhat sharply contrasted, is well illustrated by comparing HALDANE's subject, "Can evolution be explained in terms of present known genetical causes?" with the heading under which I chose to speak, "The evolutionary modification of genetic phenomena." My own address might equally well have been entitled, "Can genetical phenomena be explained in terms of known evolutionary causes?" The one approach, as you perceive, is analytic and deductive. Genetic studies are regarded as revealing the mechanism connecting cause and effect, from a knowledge of which the workings of the machine can be deduced and the course of evolutionary change inferred. The other approach is inductive and statistical; genetics supplies the facts as to living things as they now are, facts which, like the living things in which they occur, have an evolutionary history and may be capable of an evolutionary explanation, facts which are not immutable laws of the workings of things but which might have been different had evolutionary history taken a different course.

I can only discuss a small portion of the subject. Genetic phenomena concerning the chromosomal organization, such as the male haploidy of the social hymenoptera, as SNELL has suggested in an illuminating paper recently published in the *American Naturalist,* may have an adaptive significance; and I think we may look forward with confidence, as the facts become better and more systematically known, to discovering the significance of such phenomena as male linkage in Drosophila, and of the marvelously intricate chromosomal mechanism which is being unraveled by METZ in Sciara. The only two phenomena I can attempt to touch upon are those of dominance and linkage; and on these I can only put before you a selection of the facts (many of which I owe to the kindness during the last few days of other members of this congress) which seem to me to supply a good deal of light and guidance in forming an interpretation of the general body of genetic facts. As I am a mathematician by trade perhaps I should explain that I shall use no mathematics, partly because I recognize that the first duty of a mathematician, rather like that of a lion tamer, is to keep his mathematics in their place, but chiefly because I think that mathematics, though well fitted to elucidate detailed points of special intricacy, are after all only a special means of carrying out reasoning processes common to all scientific work, and are out of place in a theory covering a wide range of disparate phenomena. I believe that no one who is familiar, either with mathematical advances in other fields, or with the range of special biological conditions to be considered, would ever conceive that everything could be summed up in a single mathematical formula, however complex. If I am tempted for brevity to express myself in generalizations, it is not because I think exceptions are unimportant. One of the things about them that is important is that they are exceptions; and it seems to me that it is only by obtaining an understanding of the body of cases which constitute the rule that we can usefully hope to investigate the special causes which have produced an exception.

Dominance modification is a special case of the general fact that the expression or manifestation of a genetic factor, or gene substitution, is conditioned by the genotype in which the substitution is made. Phrases such as epistatic factors, duplicate factors, complementary factors, etc., showed an early recognition of some special cases of this general fact, which has I believe impressed itself more and more on the minds of geneticists, just in proportion as their work has become more detailed and more thorough. If the interaction of factors affects principally the heterozygote, then the relationship which we call dominance will be affected. For example, since all knowledge naturally starts with Drosophila, the dominant mutant Gull, found by MOHR in the second chromosome, is, like so many dominants, lethal when homozygous. The recessive dachsous suppresses Gull in the heterozygote, while the homozygote remains lethal. In the presence of dachsous, therefore, Gull is a recessive lethal, although without dachsous it is quite an ordinary dominant. Drosophilists could probably supply more than one parallel. Here is one from poultry. Frizzle is a dominant which curls the feathers out in a peculiar manner. The homozygote is viable though delicate through losing much of its plumage. Both LANDAUER and HUTT have a recessive mutant which largely suppresses the frizzle effect in the heterozygote, with only occasional or little effect in the homozygote. The suppressing factor by itself seems to be undetectable except by its effect in shifting frizzle some way toward recessiveness. Now dachsous is presumably injurious to survival in wild conditions, and the same may be true of the modifier of frizzle, though there is no evidence for this; but it is clear that, whatever effect the modifier may exert by itself, yet in a population descended from an ancestry containing any perceptible proportion of heterozygous frizzles, its interaction with frizzle would have given it an increased frequency of survival and have tended to make it spread through the population. The magnitude of this tendency depends chiefly upon the frequency of heterozygous frizzles in the ancestry, and this in turn must have depended on the mutation rate by which the frizzle gene was produced and on the viability of the heterozygous frizzles. It is easy to see that the viability of the homozygous frizzle and the effect of the modifier upon it are unimportant items of the calculation. As a typical case, one may take a mutation rate of one in a million in each generation and see how the proportion of heterozygous frizzles in the ancestry depends upon their viability compared to the normal non-frizzle birds. For 99 percent viability the proportion is about one in five thousand, for 90 percent about one in sixty thousand, for 50 percent about one in seven hundred and fifty thousand. The point of this simple calculation is to show that the rate of modification depends very greatly on the level of viability already attained. A seriously handicapped heterozygote will be modified very little indeed, even in periods of time ample to bring a more viable heterozygote up to complete normality. The course of the evolutionary progress of the heterozygote will be a rising curve—the later stages of its modification being much more rapid than the earlier.

When a heterozygote has been modified up to complete normality, the factor appears as a recessive; if the homozygote happens to be lethal all progress would seem to have ceased, and we should expect to find, as indeed we do find, an enormous number of mutations hung up in the uninteresting condition of being merely recessive lethals. If, however, when this stage is reached the homozygote is viable, a second stage of progress will commence, directed this time to the improvement of the homozygote and depending as to its speed on the viability of the homozygote just as the first stage of progress depended on the viability of the heterozygote. Examples of the modifiability of the homozygote are almost too abundant. I must however mention, for the sheer beauty of their demonstration, the group of recessive suppressors of vermilion, sable, black, and purple, the existence of which was first suspected by BONNIER, which have been shown by BRIDGES and SCHULTZ to be certainly not duplications, as was at first believed. In the presence of the suppressor, the vermilion homozygote is normal, and the vermilion mutation is, as far as is known, undetectable. Whereas the first stage of modification ends in a recessive condition with a lethal, or viable and recognizable, homozygote, the second stage reduces it to a state of obliteration, from which it can only be made to appear as a specific modifier if it happens to be a sufficiently substantial modifier of any mutant which is being studied.

It is important to consider how frequently these processes are actually occurring and how generally we should expect that the condition observed is a stage in a process of continuous modification. The examples I have given of known modifiers have necessarily been factors having a relatively large and regular effect. The study of quantitative characters, however, or of peculiarities having variable manifestation, seems invariably to show evidence of a numerous group of modifying factors having each only a slight effect. The cases in which new mutants are found to be affected by a fluctuating variation having a hereditary basis are very numerous; frequently the mutant type has been found to be modified perceptibly toward the wild-type by the natural selection of modifiers mitigating its expression in competition in the conditions of culture. For this reason I am inclined to think that the large modifiers, such as those which suppress the whole manifestation at a single step, have not been the principal agency of dominance modification in the past history of the species studied. In particular, there are reasons for thinking that the homozygote, on the modifiability of which most of our experience is based, has been modified considerably more slowly on the average than the heterozygote. In *Drosophila melanogaster* the mutants classed as recessives with viable homozygotes are about sixteen times as numerous as the semi-dominants with viable homozygotes. These dominants, being incomplete dominants, may be regarded as being still in the first stages of modification, and the recessives, or at least those of them in which the recessiveness is really complete, must be in the second stage; their relative numbers suggest as an upper limit that the homozygote may take on the average sixteen times as long as the heterozygote to complete the normalizing process. The largest factor in causing this difference is, I imagine, that the homozygotes probably commence their modification at a lower viability than the heterozygotes, for, as I have shown, a moderate difference in viability may greatly retard the rate of selective modification.

The possibility of modification of dominance by genetic substitution is, I suppose, now unquestioned; but the conclusion that the condition of dominance now observable is in any case the result of evolutionary modification is an inference subject, like all such inferences, to some such proviso as "unless some unknown cause prevents the process." This is a proviso to which all evolutionary theory is necessarily subject. SEWALL WRIGHT, if I understand him, has suggested that there is such an obstacle and that very small selective intensities do not, as one would naturally assume, exert effects proportional to their magnitude; but I have so far found it impossible to set up any reasonable scheme of genic interaction which would justify this conjecture. The fact of the evolutionary modification of dominance is, however, demonstrated by HARLAND's case of the mutation known as crinkled dwarf in the new world cottons. This mutant is of frequent occurrence in Sea Island cotton and some of its derivative varieties, but has not been found in large selfed progenies in the Upland group. As crosses seemed to indicate that the dominance relationship was modified HARLAND has introduced crinkled dwarf by five generations of backcrossing into the Upland species and has shown that in that species it is an incomplete dominant. The evolutionary process by which these two species have been differentiated has therefore included the modification of their reaction to the crinkled dwarf mutation in such a way that in the species in which it occurs the mutant has become recessive. The case indicates that whatever the cause of the modification may be it is conditioned by the appearance of the mutant in the ancestry of the population concerned, and that the means of modification is the establishment of a group of modifying factors and not merely a modification of the normal allelomorph at the locus of the mutant.

In the case of deleterious mutants the proportion of heterozygotes in the ancestry of the population must generally be small and the process of modification correspondingly slow. With species polymorphic in the wild condition, the heterozygotes for the factors determining the polymorphism are much more abundant, so that in these cases rapid modification is possible. In such polymorphic species, moreover, the mere maintenance of a stable gene ratio requires that the selective actions must be balanced, and its stability requires that the heterozygote must generally be at a selective advantage compared to both homozygotes. The dominance relationships in such cases should be entirely different from those of the simple elimination of a recurrent deleterious mutation. I have only time for one example, where the selective balance is evidently due to opposite action in the two sexes. In *Lebistes reticulatus* WINGE has found numerous Y-linked genes affecting the spots and patches of color on the male fish. Some of these have been found to cross over into the X chromosome. These are all without manifestation in the female, apart from intersexes. The effect on the male can be seen to be dominant, since the phenotypic expression is the same whether the variant gene is in the X or in the Y or in both. There is also an autosomal gene zebrinus which is completely dominant in the male but which has shown occasional manifestation in the female when homozygous. In the female, therefore, it has an occasional recessive manifestation. These rather exceptional phenomena conform with remarkable exactness to what would be expected if the genes responsible for polymorphism are advantageous in the male and disadvantageous in the female. First, we should expect the variant genes to become dominant in the male, and recessive in the female fish. In the next stage we should expect the entire obliteration in the female of the effects of those genes which are capable of crossing into the X chromosome. Thirdly, counter-selection in the females should make the variants rarer in the X than in the Y in wild populations, whereas without selection crossing will equalize the ratio, or indeed reverse it, if AIDA is right in suggesting that crossing over from Y to X is more frequent than from X to Y. Fourthly, favorable selection in the Y with counter-selection in the X would favor those genotypes in which linkage was closest with the sex determining portion of the Y chromosome, and may thus have built up the closely sex-linked system which is observed. There is, one might think, an evolutionary opportunity for a translocation which would put zebrinus into the Y chromosome. The sex linkage, however, need not be ascribable entirely to translocations, for it is obvious that mutations that occur from the first in the Y chromosome will have the highest probability of establishing themselves in the polymorphic system. On the whole, it is difficult to see how WINGE's findings could suggest more strongly than they do the modification of both dominance and linkage in the evolutionary process.

The view of the selective modification of dominance is thus able to reconcile such contrasting facts as the prevalence of recessiveness among recurrent mutations exposed to counter-selection with the prevalent dominance of the variant forms in polymorphic species, although of these I have had time to discuss only one case. Cases where dominance is imperfect or absent are equally instructive. I will mention five classes of these: (A) In multiple allelomorphic series the heterozygotes with the wild-type gene will have occurred with sensible frequency in the population's ancestry, and accordingly the wild-type is generally dominant, but the heterozygotes of two mutant genes will have occurred scarcely more frequently than the homozygotes and should therefore show incomplete dominance. (B) As has been pointed out by FORD, DOBZHANSKY has shown that the mutants of the white eye series of *Drosophila melanogaster,* as well as sooty and ebony, while recessive in their major morphological features, are yet incomplete dominants in their small but constant effects on the shape of the spermatheca, a feature which one would expect to be unaffected by natural selection. (C) HARLAND's case in cotton shows a recessive in one species which is an incomplete dominant in a species in which it has not been exposed to counter-selection. (D) A very large number of cases could be cited in which genes that are recessive in the wild-type are incomplete dominants in artificial genetical combinations which do not occur in nature. (E) The same thing is shown by unnatural environmental conditions such as exposure of mice to X-rays until the hair falls out, when the regenerated coat in heterozygous albinos, but not in mice homozygous for color, shows patches of white hair. All these five groups of evidence, which I have not time to amplify, indicate that the relationship of dominance is usually conditioned by selection of the heterozygote, and by selection in the special genetical complex and in the special environmental conditions which exist in nature.

The theory of the evolution of dominance, like other mutations, is itself liable to modification. It must, I suppose, be subjected to an evolutionary process, and if it is found to be deleterious it also may end in obliteration. The most promising modifications may perhaps be stated very briefly in terms of the magnificent series of multiple allelomorphs of the vestigial series which MOHR put before us on Friday. First, there are one or two allelomorphs like nick which have no visible effect even when homozygous but which may be detected by a slight manifestation in heterozygosis with vestigial. On my own view the natural interpretation to put on nick would be to regard it as having already reached the stage of complete obliteration. Now HALDANE has put forward a theory of dominance modification which he thought might be more effective than mine and which depends on selection among a multitude of normal allelomorphs of different strengths, by which those are selected which completely dominate the deleterious mutants of the series, such as vestigial. On this view nick might be regarded as one of a group of normal allelomorphs which are incapable of giving a completely wild-type development in the presence of heterozygous vestigial. I think this possible selection among multiple allelomorphs may, in some other cases, be of great importance, though generally speaking selection of multiple factors is, I believe, considerably the more powerful agency. In the present case the chief difference between the two theories is that HALDANE would regard nick, or other allelomorphs like it, as having been formerly widely diffused in the wild population and as having been displaced in competition with the wild allelomorph now prevalent, owing to the inferiority of its heterozygote with vestigial; whereas I should say it was incompletely dominant to vestigial just because it had never been sufficiently widely diffused in the wild population for its heterozygote with vestigial to have been modified up to normality.

At low temperatures the effects of some of these slight allelomorphs such as pennant, PLUNKETT tells me, are enhanced, so that in cultures developed at a low temperature homozygous pennant will show a slight manifestation. I imagine that this may be such a case as MULLER had in mind in suggesting that dominance might have been acquired as a by-product of the wild-type gaining stability of manifestation under variable environmental conditions. This modification of my views differs from HALDANE's in relying on multiple factors rather than on multiple allelomorphs, while, on the other hand, it differs from us both in that dominance in MULLER's view would be acquired without the previous prolonged occurrence of mutations of the vestigial series. I believe this view would account for the continued progress of pennant toward obliteration until it is unrecognizable even at the lowest temperature possible. I do not yet see how it accounts for the fact that the heterozygous vestigial more closely resembles the wild than the mutant homozygote.

In speaking of the modification of the results of single mutations, I implied that the rate of modification would be negligible for forms having less than 50 percent viability in the wild conditions, and that the lethal forms would be unmodifiable. In such a series as has been found at the vestigial locus, such a static and pessimistic view seems unwarranted. The members of the series that, while not completely normal, have yet a high viability are doubtless exerting a relatively strong selective action on the modifiers available, and these same modifiers are doubtless in some measure simultaneously improving the viability of all other members of the series. As I judge the situation, they must, as the song says, "all go the same way home," and though some, no doubt, would be quite incapable of progress if left to themselves, yet it would seem that their more viable companions must help them along. Even a lethal is not necessarily beyond such assistance but might be hoisted out of the ditch if the others are numerous and active enough.

Thesis approved for the degree of Doctor of Science in the University of London.

THE POSSIBILITIES OF AN INTERNATIONAL SYSTEM FOR THE CLASSIFICATION OF SOILS

BEING A CONSIDERATION OF

THE INFLUENCE OF GEOLOGY AND CLIMATE ON SOIL TYPES. A COMPARATIVE STUDY OF SOUTH-EAST ENGLAND AND CENTRAL NEW JERSEY, U.S.A.*

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* This paper is virtually the thesis presented by the author for the degree of D.Sc. in the University of London, June, 1931, now revised for the press. The thesis has the title here printed in three lines of small capitals.

† It seems appropriate that this memoir should be published at Wye where the monumental work of Hall and Russell (1911) on the soils of South-East England was begun. With his headquarters at Rothamsted it is not surprising that working in Kent the author should find co-operation at Wye and facilities for his work; it is with pleasure that we record our keen appreciation of the association of Prof. Linwood L. Lee with the pedologists at the South-Eastern Agricultural College.—EDITOR.

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I. INTRODUCTION.

THE interest of man in the soil and soil types dates back many years before the Christian era. M. P-H. Lee shows that even as early as 2357 to 2261 B.C. in the Yao dynasty, according to early Chinese records, studies of the soil classification were in use. The early Greek and Roman philosophers as represented by the writings of Cato, Columella and Pliny showed very intimate knowledge and interest in the soil. Jariloff (1901) has shown that in the seventh century before Christ, soil descriptions were in existence. The interests of the ancients were confined, however, rather to the use of the soil in the production of the crops, no attention being paid to the study of the characteristics of the soil itself.

About the beginning of the nineteenth century, earnest scientific interest in the field study of soils and soil classification began, and in these early investigations we find the beginning of Pedology as we understand it. This modern science is now defined as "that branch of soil science which treats of soil formation and development, soil classification and the characteristics of soils in their natural position as a definite portion of the earth's crust." Pedology is therefore now recognised as a distinct branch of soil science (Edaphology). An examination of early pedological literature shows a great diversification of the subject matter; of special interest and as indicative of the early concept of the soil are the various definitions of the soil itself. One of the earliest, offered by Carl Sprengel (1837) designated the soil as "a comminuted and changed mass of material derived from minerals, containing the decomposition products of plants and animals." Friederick Fallou (1855) defined the soil as the product of weathering, which gnaws into the hard crust of the earth like teeth and gradually destroys its solidity. G. Berendt (1877) called the soil the weathered hull or shell of the existing earth's surface which comes in contact with the air. Later, Dokuchaiev (1886) defined the soil as the layers of material lying on the surface of the earth or near it which have been changed by natural processes under the influence of water, air and living and dead animal matter. The modern ideas of Russian Pedology find their beginning in the teachings of this early pedologist. Hilgard (1914), one of the first of early American soil investigators, called the soil "the more or less loose and friable material in which, by means of their roots, plants may or do find a foothold and nourishment as well as other conditions of growth." Tulaikoff, of the Russian School (1908) defined the soil as follows: "By the word 'Soil' we mean the loose surface of the earth's crust in which general dynamic processes have taken place, and are taking place in conjunction with chemico-biological processes." Shaw, et al. (1927) offered the following definition: "The soil is a natural body, having a definite morphology, developed by the forces of weathering from inorganic and organic materials, and occupying the surface of the earth's crust." This might well be accepted as the most recent and modern definition of the soil.

A review of these varied ideas very clearly indicates that there have been two generally held concepts of the soil, one of which usually defines the soil as "a mixture of broken and weathered fragments of rock and decaying organic matter" or employs similar terms, while the other and modern concept reflects the work of the early Russian school, defining the soil as "a natural body quite apart from any other object or group of objects and having its own morphology." It is also noteworthy to state that it is largely through the influence and vast work of the Russian soil scientists since Dokuchaiev's time that modern soil science and pedology have developed as separate and distinct sciences quite apart from geology, chemistry, and mineralogy with which they were so long associated.

II. METHODS OF SOIL CLASSIFICATION.

Field studies in soils suggested the necessity for a system of soil classification and many schemes have been advanced to meet this need. Research workers early recognised that it was as necessary to have a classification for soils as for any other group of natural objects in order that systematic and thorough investigations might be pursued in soil science.

1. GEOLOGICAL.

Many of the early methods of classification were based on the geological factor that is, on the relation of the soil to the geological materials from which it was derived. Interest was also early shown in the construction of maps showing the geologic and soil materials in various regions. Lister in 1683 first proposed to the Royal Society of London the making of a map to show materials composing the surface of the earth. In 1743 Packe executed a map of Kent showing the occurrence of minerals. Fuchsel in 1773 and Gloser in 1775 first used colours to show granite, limestone and so on, and their work may be regarded as the first real geologic map in the modern sense. Interest in geologic and soil classification then seemed to lag and it was not until 1864-67 that, according to Blanck (1911), the first soil map in Europe was prepared by B. Forder of Halle. This work was, however, preceded in 1863 by the work of M. S. Gras who produced a map of the Department of Isere showing the geology, agricultural soils, altitude of agricultural areas, and culture of the region. Coffey (1911) reports that in 1820 the Geological Survey of Albany, New York, published a classification of the soils of that district. It was noted that the soils of alluvial formations were especially of high value. In this paper, soils were designated (1) Alluvium and (2) Geest (unmoved soil) and were classified as (a) granulated soil, (b) hardpan, (c) upland loam, (d) upland clay and (e) lowland loam. Coffey (1911) also refers to the work of Edward Hitchcock in Massachusetts (1841) as the first map in the United States which claimed to show the different characters of the soils of any district. This was a geological map, but also gave fourteen groups of soils, each of which was further sub-divided. In many of the different states of the United States, geological surveys published between 1820 and 1890 gave descriptions and generalised groupings of the soils of different regions. In Kentucky, Mississippi, Alabama and Wisconsin, for example, work of this type was carried on and several other states could be cited.

It is, therefore, quite evident that many early attempts at soil classification and the construction of soil maps both in Europe and the United States were largely along geological lines. This is a natural development when it is considered that most of the early students of soils were geologists, or men with special training in geology.

2. ECONOMIC: ACTUAL OR POTENTIAL FERTILITY.

Hazard proposed a system of classification based upon the economic factors controlling the crops grown, and with assessable land valuations. Others have also proposed similar systems. Such a classification includes however so many other factors as to take it outside the range of a scheme of soil classification, and this proposed system has not up to the present been utilised to any considerable degree anywhere in the world.

3. NATURAL VEGETATION.

In the United States, Hilgard (1914) in Mississippi pointed out the close correlation existing between the type of native vegetation and the productivity of the soil. In the

Tenth Agricultural Census of the United States as a part of the report on Cotton Production, Hilgard includes an agricultural map of the cotton producing states, giving also chemical analyses of selected soils of important areas and designating the regions largely upon the characteristics of the tree growth. Other workers have also shown relationships between certain types of vegetation and single characteristics of the soil such as Hydrogen ion concentration or soil texture. The early settlers of America were no doubt able to gain much practical information about the nature and producing power of the soil from the observations of native trees and other vegetative growth. Apart from the above references, however, the literature fails to show any further attempts to utilise classifications of this type.

4. GEOLOGIC AND AGRICULTURAL.

A somewhat different problem involving the field study and survey of soils with some detail was handled by Hall and Russell (1911) in mapping an area in Kent, Surrey and Sussex, England. The area is said to be " roughly 100 miles long and 50 miles wide," and maps of the Geological Survey were used. Mechanical and chemical analyses of the various soil types were made and records of crops grown, as well as of cultural practices studied, were included. The objects were: (1) To show the distribution of soils of similar agricultural properties and to define these soils by some method of analysis. (2) To trace such correlations as exist between the chemical and physical properties of the soils and the crops and agricultural methods that are actually associated with them. (3) On the basis of the observed distribution of the soil types and the ascertained associations, to afford guidance as to cropping and manuring over the whole area. The authors found that in this area the geologic drifts were quite uniform and that soil boundaries for the most part coincided with boundaries laid down on the geologic map. Analysis of numerous composite samples from the formations showed considerable uniformity in texture but rather wide variation in chemical constituents except as regards organic matter. Temple (1929), no doubt influenced by this work of Hall and Russell, has published a similar very creditable survey of Buckinghamshire, England.

5. PHYSICAL CLASSIFICATION.

From time to time Thaer published several soil classifications. In the first of these he distinguished twenty varieties of soil to which were given the following names: Argillaceous, Sticky Humus (two varieties), Strongly Marly, Loose Humus, Sandy Humus, Heavy Clayey, Marly, Clayey, Loamy (four varieties), Loamy Sandy (two varieties), Sandy Clayey (two varieties), Sandy (three varieties). This classification is essentially a physical one and was later enlarged upon by Thaer himself. It never found general application in Pedology.

6. PETROLOGIC CLASSIFICATIONS.

Fr. Alb. Fallou (1862) suggested a rather detailed system of classification upon petrographic grounds in which soils were grouped according to their petrologic origin. Richthofer (1886) published a more detailed system of classification also based largely upon petrologic grounds. Hendrick and Newlands (1928) have more recently reported upon similar ideas.

7. CHEMICAL CLASSIFICATIONS.

Knop (1871) offered a chemical system of soil classification in which soils were placed in one of three chemical major groups (silicates, carbonates, sulphates). Much similar work has from time to time been published but no satisfactory system of chemical classification has been favourably accepted.

8. GENERA ET SPECIES TERRARUM.

Nowachi (1892) proposed a system of Latin terminology in which the genera are based on the quality of the soil, whether stony, sandy, clayey, peaty, etc., and the species are dependent upon the quantities of organic matter and clay. The literature does not indicate that the system has been put into practice to any very great extent.

9. ILLINOIS SYSTEM.

A feeling that the United States Bureau of Soils methods of soil classification were inadequate for Illinois led Hopkins about 1905 to devise and put into practice a distinct system for that state. The soils are grouped first as to glaciated or unglaciated, and if glaciated placed according to glaciation period. They are further divided according to colour, topography and texture of soil and subsoil. This method has since been replaced by the System of the Bureau of Soils.

10. GENETIC.

One of the most interesting schemes of soil classification and one that at the present moment is receiving much consideration is that of the Russian school. It is based upon the " conception of the soil as a natural body having a definite genesis and a distinct nature of its own." Due to the present importance of this scheme of classification, a detailed outline is therefore thought advisable. According to this system the formation of natural soils include these factors : (1) Petrographic type of parent rock. (2) Nature and intensity of processes of disintegration in connection with local climate and topographic conditions. (3) Quantity and quality of that complexity of organisms which participate in the formation of the soil and incorporate their remains in it. (4) Nature of the changes to which these remains are subjected in the soil. (5) Mechanical displacement of the particles of the soil, provided this displacement does not destroy the fundamental properties of the soil, its geo-biological character, and does not remove the soil from the parent rock, and (6) Duration of processes of the soil formation.

In the Russian classification the soils are grouped in a broad way into seven zones, ranging from the Laterite soils in the tropics to the Tundras in the arctics, each of which in general correspond to a zone of climate. This zonal system of classification as recorded by Sibirceff is as follows :—

Class I.—Zonal Soils, complete.
    Type 1. Lateritic—warm humid tropics and sub-tropics.
    Type 2. Atmospheric Eolian—Continental regions of dry climate.
    Type 3. Soils of the Steppe Deserts, or dry Steppes.
    Type 4. Chernozem—Herbaceous steppes of temperate or cold climate.
    Type 5. Soils of wooded steppes and grey forest soils.
    Type 6. Sod soils and Podsol soils—cold temperatures. Iron concretions.
    Type 7. Soils of the Tundras.
Class II.—Intrazonal Soils.
    Type 1. Alkali lands.
    Type 2. Humus—Calcareous soils.
    Type 3. Marshy soils, etc.
Class III.—Incomplete or Azonal Soils.
    Soils formed *in situ*.
        (*a*) Crude—imperfectly developed soils.
        (*b*) Coarse soils.
            (Sub-Group—Alluvial soils.)

This system of classification was first advanced by Dokuchaiev in 1879 and again appeared in revised form in 1886. It was finally presented in the above form by Sibirceff and since enlarged upon by Glinka (1928) who, until his recent death, was generally recognised as the leader of the modern school of Russian soil science.

II. THE AMERICAN AND NEW JERSEY SYSTEMS.

The classification and mapping of the soils of the United States began about 1899. It owes its origin to the work of the late Milton Whitney and a little band of associates who, in this year, with very limited means and knowledge of methods and procedure, completed a series of soil surveys of 720,000 acres in various parts of the United States. The response to the publication of this survey was immediate and from that time forward the work constantly advanced. Increased financial support brought expanded personnel and experience, and, in time, more refined and accurate field methods. The present system of soil classification in the United States, as originated by Whitney in 1899 and since vastly improved by Marbut (1913-1928), is therefore the result of accumulated field experience of soil examination gained over a period of the past thirty years. The New Jersey system, while essentially based upon the American method, was developed in New Jersey by the author (1926). As the New Jersey system of classification was utilised in carrying out the field studies in New Jersey and South-East England upon the results of which this paper is based, it is deemed advisable to describe this method in complete detail.

The New Jersey Method of Soil Classification.—One of the most important principles of the New Jersey method of classification is that soils are defined and classified on the basis *of the characteristics of the soils themselves* rather than in their relationship to other things, such as geology, climate, natural vegetation, or crops. The unit of classification is the SOIL TYPE, which is a combination of a SERIES NAME and a CLASS (texture) NAME, as for example, SASSAFRAS LOAM, in which " SASSAFRAS " indicates the SERIES NAME and " LOAM " the CLASS (texture) NAME, the two names together representing the soil type.

THE SOIL SERIES.

The determination, establishment and recognition of a soil series is based upon the natural properties of the soil itself. For convenience the following soil characteristics may be employed: (I) Geological origin of the soil material. (II) Mode of formation. (III) Topographic position. (IV) Natural drainage. (V) Profile. We may now consider these characteristics in some detail.

I. GEOLOGICAL ORIGIN OF THE SOIL MATERIAL.

As the writer will show in a later section of this memoir, the mineral matter in soils falls into three groups of materials ; sands, silts and clays. These are derived from a variety of sources which may be stated broadly as follows :

(a) *Igneous Rocks.*—The classification of this great group of rocks is a matter of much complexity. Some kinds of igneous rocks are of great importance in the formation of soil (e.g. Granite) others by their rarity are quite unimportant, but each kind recognised by the geologist must be considered in detail according to local conditions.

(b) *Sedimentary Rocks.*—These include: (i) Argillaceous materials such as shales, (ii) Arenaceous sediments, including sandstones, grits and conglomerates, (iii) Limestones, both massive and earthy ; to the latter kind belongs chalk,

(c) *Metamorphic Rocks.*—These are altered rocks either igneous or sedimentary in origin and comprise such materials as gneiss, schist, slate, quartzite and marble.

(d) *Unconsolidated Deposits.*—This is a convenient term under which we may include such deposits as Plateau Loam, Coombe Rock, Gravel, Loess, Brickearth, Sand and Clay.

II. MODE OF FORMATION.

Here we consider the various agents responsible for the present situation of the mineral part of the soil. (a) The first of these is Water which has given rise to such materials as Alluvium, River-Terrace and Cumulose Deposits. (b) Ice was responsible for Glacial Drift and in part for fluvio-glacial residues. (c) To Gravity, Colluvium or Rainwash owes its origin. (d) Wind is responsible for Sand Dunes and plays its part in various other ways. (e) Those materials of the soil which have not been transported are termed Sedentary, or in the United States, Residual.

III. TOPOGRAPHY takes into consideration the nature of the surface, describing it as rugged, steep, rolling, gently rolling, sloping, level, depressed and so on.

IV. NATURAL DRAINAGE is described by such terms as excessive, good, satisfactory, imperfect, fair, impeded, poor.

V. PROFILE. This is a feature of great importance, as it includes a number of subdivisions. The arrangement of the layers, their colour, chemical composition, structure, texture, thickness and consistency are noted.

All soils having *identical* characteristics belong to the *same* soil series. The soil series is usually named from some place or natural feature near the locality where the soil is first studied. Subsequent correlation prevents repetition of series names, and soils of uniform characteristics are thus included in the same series.

THE SOIL CLASS.

The soil class is the *textural* unit of classification and is dependent upon the relative proportion of the various soil separates which make up the soil mass. In the United States* the following separates are recognised :—

Fine Gravel	2 to 1 mm.
Coarse Sand	1 to 0.5 mm.
Medium Sand	0.5 to 0.25 mm.
Fine Sand	0.25 to 0.1 mm.
Very Fine Sand	0.1 to 0.05 mm.
Silt	0.05 to 0.005 mm.
Clay	0.005 to 0 mm.

together with more or less organic matter.

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* The scale now usually employed in Great Britain is as follows :

Stones and gravel	over 2.0 mm.
Coarse sand	2.0—0.2 mm.
Fine sand	0.2—0.02 mm.
Silt	0.02—0.002 mm.
Clay	0.002—0 mm.

Organic matter in soils seldom amounts to more than 10 per cent. in agricultural soils and usually to much less. In the average soil it is usually thoroughly decomposed and falls within the clay group of separates, so that the texture of most soils depends mainly upon their mineral composition. It is also evident that the heaviness or lightness (texture) of any soil is due to the relative proportion of the various soil separates which make up the soil. Experience has shown that a great range of combinations exists and therefore in the official classification of the United States Bureau of Chemistry and Soils twenty textural groups are recognised. These groups are as follows :—

(1) *Coarse Sand.*
(2) *Sand.*
(3) *Fine Sand.*
(4) *Very Fine Sand.*
(5) *Loamy Coarse Sand.*
(6) *Loamy Sand.*
(7) *Loamy Fine Sand.*
(8) *Loamy Very Fine Sand.*
(9) *Coarse Sandy Loam.*
(10) *Sandy Loam.*
(11) *Fine Sandy Loam.*
(12) *Very Fine Sandy Loam.*
(13) *Loam.*
(14) Silt Loam.
(15) Sandy Clay Loam.
(16) Clay Loam.
(17) Silty Clay Loam.
(18) Sandy Clay.
(19) Clay.
(20) Silty Clay.[*]

If gravel, rock or shale are present in such quantities as to influence the economic value of the soil type the class name is qualified by the terms " gravelly," " stony " or " shale " respectively :—

Gravelly soil (30 per cent. or more fine, medium and coarse gravel particles or stones varying up to 4½ inches in diameter ; rounded or angular).

Stony soil (enough stones over 4½ inches in diameter to interfere with cultivation ; rounded or angular).

In the actual determination of the soil class (texture) in the field, only the surface layer, the top 6 to 12 inches, receives consideration and the class of the soil is named accordingly. The other soil characteristics are examined in the field and the " Soil Series " is established and named according to these characteristics. The series name followed by the class name, designates the individual soil, or " Soil Type " as it is called. This is the unit of soil classification.

THE LIMITS OF THE PROPORTIONS OF SAND, SILT AND CLAY PRESENT IN THE VARIOUS SOIL CLASSES.

The soils of the twenty classes of the official United States system are gathered into three major groups according to their clay content :

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[*] In New Jersey and South-East England the writer has adopted a textural classification which is a slight modification of the above and as it has found some acceptance it is given here The first thirteen classes are those printed in italics in the above list. Thereafter follow :—

(14) *Silty loam.*
(15) *Silt loam.*
(16) *Silty clay loam.*
(17) *Clay loam.*
(18) *Clay.*

Taking a large area as a whole the writer prefers the official classification, but for the special conditions of New Jersey and South-East England the modified scale is an advantage.

[†] This subject is dealt with by Bennett and Allison, 1928, in *The Soils of Cuba*, and by the present author in *Technical Communication*, No. 6. Imperial Bureau of Soil Science.

First, soils containing less than 20 per cent. clay:

(a) Soils containing less than 15 per cent. silt and clay, are SANDS, as follow :—

(1) *Coarse sand* (35 per cent. or more fine gravel and coarse sand, and less than 50 per cent. fine and very fine sand).

(2) *Sand* (35 per cent. or more fine gravel, coarse and medium sands, and less than 50 per cent. fine and very fine sand).

(3) *Fine sand* (50 per cent. or more fine and very fine sand).

(4) *Very fine sand* (50 per cent. or more very fine sand).

(b) Soils containing from 15 to 20 per cent. silt and clay are LOAMY SANDS as follow :—

(5) *Loamy coarse sand* (35 per cent. or more fine gravel and coarse sand, and less than 35 per cent. fine and very fine sand).

(6) *Loamy sand* (35 per cent. or more fine gravel, coarse, and medium sand, and less than 35 per cent. fine and very fine sand).

(7) *Loamy fine sand* (35 per cent. or more fine and very fine sand).

(8) *Loamy very fine sand* (35 per cent. or more very fine sand).

(c) Soils containing from 20 to 50 per cent. silt and clay are SANDY LOAMS, as follow :—

(9) *Coarse sandy loam* (45 per cent. or more fine gravel and coarse sand).

(10) *Sandy loam* (25 per cent. or more fine gravel, coarse and medium sand, and less than 35 per cent. very fine sand).

(11) *Fine sandy loam* (50 per cent. or more fine sand, on less than 25 per cent. fine gravel, coarse and medium sand).

(12) *Very fine sandy loam* (35 per cent. or more very fine sand).

(d) Soils containing 50 per cent. or more silt and clay are LOAM and SILT LOAM :

(13) *Loam* (from 30 to 50 per cent. silt, and from 30 to 50 per cent. sand).

(14) *Silt loam* (50 per cent. or more silt, and less than 50 per cent. sand).

Second, soils containing from 20 to 30 per cent. clay are CLAY LOAMS :—

(15) *Sandy clay loam* (less than 30 per cent. silt, and from 50 to 80 per cent. sand).

(16) *Clay Loam* (from 20 to 50 per cent. silt, and from 20 to 50 per cent. sand).

(17) *Silty clay loam* (from 50 to 80 per cent. silt and less than 30 per cent. sand).

Third, soils containing 30 per cent. or more clay are CLAYS :—

(18) *Sandy clay* (from 30 to 50 per cent. clay, less than 20 per cent. silt, and from 50 to 70 per cent. sand).

(19) *Clay* (less than 50 per cent. silt, and less than 50 per cent. sand).

(20) *Silty clay* (from 30 to 50 per cent. clay, from 50 to 70 per cent. silt, and less than 20 per cent. sand).

SPECIAL SOILS.

Certain soils are highly organic and cannot be included naturally nor satisfactorily in the above classification. Special categories have been established to embrace them as Peat, Peaty Loam and Muck.

PEAT is 65 per cent. or more organic matter, sometimes mixed with considerable sand, silt and clay.

PEATY LOAM is from 20 to 25 per cent. organic matter mixed with much sand and silt, with but little clay.

MUCK is from 25 to 65 per cent. well-decomposed organic matter, mixed with much clay or silt and some sand.

Table I shows the broadest possible ranges in content of sand, silt and clay in the ten principal soil groups.

TABLE I.

The Principal Textural Groups according to their Limits of Mechanical Composition.

	Percentage Limits of Mechanical Composition.		
	Sand	Silt	Clay
Texture (Soil Class)			
Sands	80–100	0–20	0–20
Sandy Loams	50–80	0–50	0–20
Silt Loams	0–50	50–100	0–20
Loams	30–50	30–50	0–20
Silty Clay Loams	0–30	50–80	20–30
Sandy Clay Loams	50–80	0–30	20–30
Clay Loams	20–50	20–50	20–30
Silty Clays	0–20	50–70	30–50
Sandy Clays	50–70	0–20	30–50
Clays	0–50	0–50	30–100

III. RECENT CONTRIBUTIONS IN SOIL CLASSIFICATION IN THE BRITISH ISLES.

A study of the recent literature shows that numerous soil surveys and field studies in soil classification have been completed and from time to time published in various parts of the British Isles.

ENGLAND.—Hall and Russell's (1911) work on the Agriculture and Soils of Kent, Surrey and Sussex, previously cited, is a most noteworthy contribution to early soil classification in England. Temple's (1929) survey of Buckinghamshire, also previously referred to, is much along the same lines as the publication of Hall and Russell. Wright and Ward (1929) have contributed a similar report on *A Survey of the Soils and Fruit of the Wisbech Area.* In all of these the close relationship of the soils to the geology of the districts is emphasised. Robinson has published *A Survey of the Soils and Agriculture of Shropshire* in which the relationship between the classification of the soils and the geological formation is discussed in detail. Brade-Birks and Furneaux (1928-30) working on field studies in East Kent have published a series of papers, reporting upon the nature of the soil profiles of the district and the formation of pans in podsol soils. Their conclusions are based upon the Russian genetic system of classification. These same co-authors (1930) have published in last year's issue of this *Journal* a commendable paper reporting upon their *Soil Survey of the College Farms* (*Wye, Kent*) with a detailed soil map upon which the location and occurrence of the various soils are shown by accurate boundaries. The New Jersey system of soil classification is utilised and eleven soil series are identified and described. This is the first published detailed soil map in England in the construction of which an American system of soil classification has been employed.

In reviewing all these British publications, one is at once impressed by the early recognition by a majority of the authors of the close relationship between the geological formations and the soils of the British Isles. More recent work shows the influence of the Russian ideas of soil genesis. It is also important to point out that apart from Robinson's most noteworthy contribution as outlined in his paper entitled " A Method for the Classification of Soils for Purposes of Survey," and Brade-Birks and Furneaux' " Soil Survey of the College Farms," no previous attempts have been made to name and classify actual soil types as individuals. It should also be stated that up to the present there has apparently been no system of classification uniformly accepted by the pedologists of the British Isles. It is to be hoped and certainly highly desirable that some satisfactory method be universally adapted in the near future.

SCOTLAND.—In 1928 Newlands published a work on Scottish soil types with special reference to North-East Scotland. This was a profile study after the Russian genetic system. It was concluded that " the general characteristics of Scottish soils place them in the podsolic group, but for agricultural purposes this group must be sub-divided and particular features of the profile which then become important are local variations in organic content and moisture content which are largely determined by topography, texture and chemical composition, which largely depends on the physical and mineral composition of the parent material." It was therefore concluded that the Russian system was not entirely satisfactory for the classification of the soils of this district as such factors as topography, parent material, organic and moisture content, chemical composition and texture were also recognised as of great importance.

In the same year, Ogg presented a paper on Scottish Soils in relation to climate and vegetation in which is pointed out the climatic and vegetative characteristics of the country. It is concluded that the soils belong to the podsol group, but are in many areas modified into moor and less distinct podsols by the destruction of the original forests. Parts of the South-East coast (low rainfall district) show soils which resemble those described by Ramann as " brown earths." Most of the soils show a decided acid reaction on the surface and become less acid or alkaline in the lower horizons. There is also in many places a clearly developed iron or other hardpan and in many other places the soils may be grouped on the basis of parent material.

Hendrick and Newlands, also in 1928, published a study on the " Mineral Composition of the Soil as a Factor in Soil Classification." By a mineralogical method outlined in this paper, certain Scottish and English soils were examined and it was found in general that the Scottish soils were characterised by a relatively high content of undecomposed silicates which contain large reserves of chemical bases, the English soils being found to contain little such material. It was further concluded that Scottish soils could be classified by the identification of minerals found in the soils and that such a system grouped together soils of similar parent material and origin. This method of classification is essentially geologic.

Wales.—Most of the recent work in Wales may be credited to Robinson and his associates, and in all of it the importance of the relation of the geologic materials to the soils and their classification is emphasised. Robinson has published work on " The Soils of Anglesey," Studies on the Palæozoic Soils of North Wales (1917), and in a very recent note published in *Nature* in June, 1930, entitled " A Method for the Classification of Soils for Purposes of Survey," he outlines a system of classification for the soils of Wales. In collaboration with Jones and Hughes (1928), this author has published a progress report (1925-1927) on the Soil Survey of Wales. This work sets forth in detail the methods of classification used, and here again the importance of geology is stressed. The salient characteristics of the soils of the separate areas are classified and mapped. It is pointed out that the system used attempts to classify the soils in such a way as to show with the greatest contrast their important properties from the standpoint of agriculture but at the same time attention is called to the fact that in the purely scientific study of Welsh soils other methods of classification must be used. Other methods are desirable in studying the relation of the soils of Wales to those of Europe because for those of the continent of Europe a provisional classification has already been devised. It is concluded that " The soils of Wales would appear to belong to those types produced by intense leaching in cool climates, of which the podsol soils are the example," and in the investigation of which it is necessary to study, " natural mature profiles unaltered by cultivation or other agencies."

Jones and Robinson in a publication entitled " Soil Studies on the Waste Lands of Lleyn," present a general discussion of the close examination of the relation between the soils and vegetation of Heath, Rhos, Gorse, Bog, Fen and Marsh. Moisture relationships, soil texture, and chemical soil characteristics were studied and it was concluded that " the differences in natural vegetation appear to be ultimately connected with situational factors. The nature of the soil itself appears to be of secondary importance, but the character of any soil is governed by the character of the parent material and by the processes of soil formation, which are mainly controlled by situation and climate. Allowance must also be made for the effect of natural vegetation on soil, in the case of organic matter."

Gethin Jones (1927) has presented a Preliminary Soil Survey of the Creuddyn Peninsula. In this work the Physical Features, Geology, Climate and Agriculture of the district are described and the soils classified as (1) Rocky Areas, (2) Carboniferous Limestone and Millstone Grit, (3) Palæozoic Silt Loams, (4) Alluvial Soils, (5) Wind Blown Sands, (6) Boulder Clays. Some analytical data are also presented and the author concludes that there are eight fairly well defined soil types in the district not, however, necessarily associated with a definite state of fertility and type of cultivation, but nevertheless there is some correlation between the soil and general farming practice. This paper was followed in this *Journal* in 1930 by " A Study of the Pedogenic Processes in an Area of Lower Palæozoic Shales." It describes the physical features, climate and agriculture of the region. A series of profile samples were taken in the field and studied in the laboratory. Numerous tables are given and conclusions drawn regarding the effect of the pedogenic processes upon the physical and chemical characteristics of the various soil profiles.

IV. THE AREAS SELECTED FOR STUDY.

The area in south-east England is approximately 700 square miles in size, located for the most part in the county of *Kent* and including a small part of *Sussex*. It is bounded by the English Channel on the south and east; the North Sea and the River

Thames on the north ; and on the west by a meridian of approximately 0° 44′ E longitude, running from the Thames on the north to the English Channel on the south. It has a maximum length of about thirty-six miles and a maximum width of about thirty-one miles.

In New Jersey the area selected for study is bounded on the north by the 40°28′ north parallel of latitude, on the south by the 40° north parallel, on the east by the 74° 20′ west meridian, and on the west by the Delaware River and 74° 46′ meridian. It has a maximum length of about thirty-two miles and at its widest point is about thirty-eight miles comprising in all also an area of about 700 square miles.

These two areas are separated by nearly 3,500 miles of the Atlantic Ocean and English Channel. England occupies a geographic position much further north than New Jersey, but enjoys for the most part, a much cooler climate in summer and a comparatively mild winter season. The annual rainfall, however, is much less in England than in New Jersey. Geologically, the two areas are similar in that the formations have a banded trend north-east and south-west in New Jersey and north-west and south-east in south-eastern England. They are of quite similar age, but differ somewhat in the nature and extent of the various geological materials. For example, in south-east England there are large areas of chalk, while no chalk occurs in New Jersey. The red sandstone and shale of Triassic age and some igneous rocks of the same period occur in New Jersey, but are absent in south-east England. Unconsolidated deposits are common and extensive in both areas. The physiography of the regions is quite similar, much of the land being at relatively low altitudes and extending in elevation from sea level to only a few hundred feet above it.

V. SCOPE OF THE SUBJECT.

Having explained the general climatic and geological conditions, it is now the object of this paper (1) to show that the soils of both areas may be classified after the same system, and (2) to indicate the influence of geology and climate on (a) the classification and distribution of the soils, (b) the soil series, (c) the soil class (i.e. texture), (d) the soil profile, (e) the soil reaction.

VI. METHODS OF STUDY.

In order to determine the characteristics of the soils of each region and study their relationship to the geology and climate, extensive field studies have been made of the soils in each district. The soil types, as encountered in the field, have been carefully examined, identified, named and classified according to the New Jersey system of soil classification, special attention being given to the profile characteristics of each soil type, as it is recognised that these characteristics are the physical and chemical expression of climatic features acting upon given geologic materials.

VII. DESCRIPTION AND PHYSIOGRAPHY OF THE NEW JERSEY AREA.

The New Jersey area comprises about three-fourths of Mercer County, one-half of Middlesex County, about one-fourth of Burlington and Monmouth County and one-sixth of Ocean and Somerset Counties. It is situated in west-central part of New Jersey, the lower half of the western boundary being formed by the Delaware River, and dividing line between the states of Pennsylvania and New Jersey. The city of Trenton, situated in the west-central part of the area, is thirty-three miles by rail from Philadelphia and fifty-seven miles from New York. The area is approximately a rectangle in outline, thirty-two miles from north to south, and from twenty-one to thirty-eight miles from east to west. The New Jersey area comprises parts of two distinct physiographic provinces, the Piedmont Plateau and the Atlantic Coastal Plain. The Piedmont Plateau in the north-western part, has a rather rolling to hilly topography, with steep to gentle slopes, frequently eroded. Many streams in this region have cut narrow, steep-sided trenches along the walls of which the country rock is exposed in many places. Intrusive masses of extremely resistant diabase give rise to relatively high elevations. These uplands range in height from 300 to a little more than 500 feet above sea level, or 150 to 350 feet above the adjacent country. On the lower elevations of the Piedmont region are the relatively soft red and grey sandstones and shales, together with some small areas of massive, fine-grained mudrock (argillite), which, being more resistant to the agents of weathering and erosion than the softer sandstones and shales, forms relatively high elevations. The soils of the region have been derived chiefly from these different types of rock. The greater part of the Trenton area, however, lies south-east of the Piedmont and constitutes, physiographically, a part of the Atlantic Coastal Plain. Its topography is level to gently rolling, being interrupted only in a few localities by isolated hills, which rise abruptly from the surrounding plain. The stream bottoms (valley floors) are wide, and in many places the streams are sluggish. Terraces border the Delaware River and some of the larger creeks. The elevation of the area ranges from sea level along the Delaware River on the west and near the town of South River in the extreme north-east to 560 feet on Sourland Mountain, near Hopewell, in the north-western part.

Streams are numerous and the waters of all of them eventually reach the Atlantic Ocean. The Delaware River is the most important and with its numerous tributaries drains more than half the area. Millstone and South Rivers are next in importance and furnish drainage outlets for the northern portion. Crosswicks Creek, in the central part of the area and Assanpink Creek farther north, both flowing into the Delaware, are important streams. Rancocas Creek drains the extreme south-western part of the region. The area as a whole is well drained except in the extreme south-eastern part, where the streams are sluggish and bordered by rather wide swampy areas. The flat level surface of this region further retards surface drainage. Bear Swamp near Lawrence, and Pigeon Swamp north-east of Monmouth Junction, are other rather important poorly drained areas.

VIII. THE GENERAL GEOLOGY OF CENTRAL NEW JERSEY.

The geological formations of New Jersey as a whole, range in age from Pre-Cambrian to Post-Tertiary and are represented by a great variety of igneous, metamorphic and sedimentary rocks. As shown by Table II, in the area studied, the rocks are of Triassic, Cretaceous, Tertiary and Post-Tertiary age, and consist of both consolidated and unconsolidated materials, mostly the latter. Structurally, the sedimentary Triassic deposits are chiefly monoclinal, dipping at low angles to the north-west, while locally shallow folds have been developed together with many vertical normal faults.

The Geological Formations of the New Jersey Area.

Triassic Rocks.—The Triassic rocks of the New Jersey area occur in the north-western part. The Brunswick formation is the most extensive and important of this group and consists chiefly of soft red shales with interbedded fine grained sandstones of the same colour. It is underlain by the Lockatong beds, made up of black shales, flagstones and hard massive dark argillites. The igneous flows are dense hard trap consisting of extrusive basalt and intrusive diabase.

TABLE II.

Classification of Geological Materials in Central New Jersey.

SEDIMENTARY ROCKS.

POST-TERTIARY.

Recent—
- Existing swamps and marshes and river flood plains.

Pleistocene.
- Cape May Formations.
- Pensauken Formations.
- Bridgeton Formations.

TERTIARY.

- Beacon Hill Gravel.
- Cohansey Sand.
- Kirkwood Sand.
- Shark River Marl.

SECONDARY.

Cretaceous.
- Manasquan Marl.
- Vincentown Sand
- Hornerstown Marl.
- Tinton Loam.
- Red Bank Sand.
- Navesink Marl.
- Mount Laural Sand.
- Wenonah Sand.
- Marshalltown Formation
- Englishtown Sand.
- Woodbury Clay.
- Merchantville Clay.
- Magothy Formation.
- Raritan Formation

Triassic.
- Brunswick Formation.
- Lockatong Formation.

IGNEOUS ROCKS (*of Triassic Age*).

- Basalt Flows and Dikes.
- Diabase (intrusive).

Cretaceous and Tertiary Rocks.—The Cretaceous and Tertiary rocks adjoin the Triassic formations on the south-east and consist entirely of unconsolidated deposits of clay, sand and greensand (glauconite). While the various deposits have been classified geologically into many formations a detailed description is not deemed necessary, as they are for the most part extensively covered to a depth of many feet by later Pleistocene materials. Generally; however, the Cretaceous formations contain a higher percentage of silt and clay than those of the more sandy Tertiary deposits, and also may contain greensand in varying quantities.

Pleistocene Deposits.—These deposits consist of gravel, sand and some clay and have a widespread distribution throughout the central New Jersey area, with the exception of the north-western portion, capping in most places to a considerable depth the underlying Cretaceous and Tertiary formations. Due to their broad distribution they have played a very important part in the formation of the soils of this region.

Summary of Geology.

The New Jersey area therefore consists in the north-western part of consolidated rocks (sandstone, shale and igneous materials) while the greater part, lying to the south-east consists of nearly horizontal beds of greensand, clay and sand, superficially covered nearly everywhere with layers of gravel, sand and clay of relatively recent geologic age. The various formations extend mostly as bands in a north-easterly south-westerly direction.

IX. THE CLIMATE OF CENTRAL NEW JERSEY.

The climate of the central New Jersey area is characterised by rather cold winters and moderately warm summers. Snow sometimes covers the ground for several weeks in winter, but extremely low temperatures are not of long duration. The ground usually

TABLE III.

Normal Monthly, Seasonal, and Annual Temperature and Precipitation at Trenton, Mercer County.

Elevation—190 feet.

Month.	Temperature.			Precipitation.			
	Mean	Absolute Maximum.	Absolute Minimum.	Mean.	Total Amount driest year.	Total Amount wettest year.	Snow average depth.
	°F.	°F.	°F.	ins.	ins.	ins.	ins.
December ..	34.4	70	− 7	3.16	4.64	2.03	3.4
January ..	30.5	72	− 8	3.17	2.72	4.40	7.2
February ..	30.7	72	−10	3.31	3.00	3.36	10.2
Winter ..	31.9	72	−10	9.64	10.36	9.79	20.8
March ..	39.1	86	6	4.04	3.28	5.67	3.8
April ..	49.8	93	21	3.29	2.57	5.00	.2
May ..	61.1	99	33	3.52	1.98	4.47	.0
Spring ..	50.0	99	6	10.85	7.83	15.14	4.0
June ..	69.5	98	41	3.49	1.74	2.67	0
July ..	74.5	101	50	4.77	4.75	9.86	.0
August ..	73.0	105	46	5.37	1.63	7.23	.0
Summer	72.3	105	41	13.63	8.12	19.76	.0
September ..	66.9	97	35	3.59	.41	10.13	.0
October ..	55.6	89	28	3.41	1.74	4.66	.0
November ..	44.4	78	10	3.43	1.62	7.75	1.1
Fall ..	55.6	97	10	10.43	3.77	22.54	1.1
Year ..	52.5	105	−10	44.55	30.08	67.23	25.9

freezes a foot or more. Although the summer months are warm, temperatures above 100 degrees F. are rare and of short duration. Late frosts in the spring often cause injury to early blooming fruits, and during more severe winters the grain, clover, and grass are in danger of being winter killed, especially on the heavier soils. As a whole, however, climatic conditions are very favourable for successful farming.

On the average the rainfall is abundant and well distributed throughout the year, the monthly rainfall averaging about 3½ inches for each month except July and August, in which months it is greater. If, however, individual years are considered, great variations in the monthly rainfall are found, and excessive rains or extreme droughts may occur in any month. The summer rainfall (June, July, August) averages 30 to 35 per cent. more than that of the other seasons, and is due primarily to sudden and heavy thunderstorms. Summer droughts occasionally cause serious damage to crops.

Table III on previous page gives complete climatic data for the city of Trenton and is fairly representative of climatic conditions throughout the area.

X. SOILS OF THE NEW JERSEY AREA.

The soils of the New Jersey area have a characteristically belted distribution. There are about four rather well defined north-east south-west belts, each passing out of the area unchanged and known to continue for considerable distances beyond this area without any fundamental change in character. The area under study, therefore, is merely a part of a broad region whose features extend across the arbitrarily bounded area designated.

The most south-easterly belt covers the south-eastern corner of the area. This is predominately a belt of sands. The Sassafras and Lakewood are the most important and extensive soil series occurring on the well drained uplands.

The Sassafras sand is grey at the immediate surface, with more or less dark stain due to organic matter. Underlying the thin grey or dark grey surface layer is a pale yellowish layer ranging up to somewhat more than a foot in thickness. This in turn is underlain by a faint reddish-brown layer, which may be slightly heavier than the one above it, but the percentage of fine material is very small. These three layers constitute the layers of the true soil, the " Solum," and are underlain by the parent geological material, which usually consists of greyish to yellowish sands. Beds of heavier materials, clays and silts, are found at considerable distance below the surface, but are at too great a depth to influence the character of the soils. The following particulars are given from field observations of a virgin profile of the Sassafras sand, two miles south-east of Smithburg.

Horizon.	*Depth.*
A_0	0"– 1"—very dark grey to very dark brown leaf mould containing a little sand—loose—acid.
A_1	1"– 3"—grey sand grading into yellowish-grey sand—loose—acid.
A_2	3"– 8"—greyish-yellow sand—loose—acid.
A_3	8"–16"—yellow to light coloured orange-yellow—loose sand—acid.
B_1	16"–20"—yellowish-brown sand—slightly loamy—loose—acid.
C_1	20"–36"—yellowish-brown sand containing lenses of gravel—loose—acid.

Aside from the colour changes it was very difficult to detect any other distinct differences in the various horizons.

Topography—Level to very gently undulating.

Native Vegetation—Scrub Oak, Chestnut Oak, Scrub Pine, Huckleberry.

TABLE IV.

Key to the Classification of the Soil Series of Central New Jersey.

Geological Material.	Mode of Formation.	Topographic Position.	Drainage.	PROFILE.								
				UPPER HORIZONS.				LOWER HORIZONS.				
				Colour.	Texture.	Consistency.	Reaction.	Colour.	Texture.	Consistency.	Reaction	Series Name.
Pleistocene, Tertiary and Cretaceous sands, clays and gravels.	Sedentary.	Level to rolling.	Good to excessive.	Greyish-brown to brown.	Light to fairly heavy.	Mellow.	Acid.	Reddish-brown to reddish-yellow.	Light to fairly heavy.	Mellow and friable.	Acid.	Sassafras
"	"	"	"	Grey to whitish.	Light.	Loose.	"	Reddish-yellow.	Light.	Loose	"	Lakewood
"	"	Depressed.	Poor.	Black.	Light.	Loose.	"	Grey over light rusty-brown	Light.	Compact cemented hardpan	"	St. John's.
"	"	Level to rolling.	Good to excessive.	Brown to greenish shades.	Light to fairly heavy.	Loose.	"	Reddish to yellowish-brown-greenish shade.	Light to fairly heavy.	Mellow to slightly waxy.	"	Collington
"	"	Slightly depressed.	Imperfect.	Brown to light brown	"	Mellow.	"	Mottled grey, yellow, bluish-grey	"	Mellow.	"	Woodstown.
"	"	Depressed.	Poor.	Grey.	"	"	"	"	"	Loose to mellow.	"	Elkton.
"	"	"	"	Black.	"	"	"	"	"	"	"	Portsmouth.
"	"	Level to rolling.	Good.	Brownish-red to red.	"	"	"	Dark reddish-brown to red.	"	"	"	Colts Neck.
"	"	Depressed.	Imperfect.	Brownish-grey to grey	"	"	"	Mottled grey, yellow, brown, green.	"	"	"	Shrewsbury.
"	"	"	Poor.	Black.	"	"	"	Mottled yellow, green, red.	"	"	"	Keansburg.
"	"	Level to rolling.	Imperfect to good.	Greyish-brown to brown	"	"	"	Mottled yellow, grey, brown.	Very heavy.	Plastic and stiff claypan.	"	Keyport.
"	"	Level to slightly depressed.	Poor.	Grey to whitish.	Light	Loose.	"	Grey over brown to rusty brown.	Light.	Compact cemented hardpan.	"	Leon.
Triassic red sandstone and shale.	"	Level to sloping.	Good to excessive.	Red.	Heavy.	Mellow to plastic.	"	Red. Bed rock may	Heavy. occur at any	Somewhat compact. depth.	"	Penn.
Grey shale and Argillite.	"	"	Good.	Brown.	"	Mellow.	"	Brownish-yellow.	Heavy.	Mellow.	"	Lansdale.
Triassic sandstone and shale.	"	Level to depressed.	Poor.	Greyish-brown to grey.	"	Stiff to plastic.	"	Reddish-brown mottled grey and yellow.	"	Compact clay pan	"	Croton.
Triassic trap rock.	"	Level to steep.	Good.	Reddish-brown.	Heavy, frequently gravelly and stony.	Mellow.	"	Reddish-yellow. Bed rock may	" occur at any	Open. depth.	"	Montalto.
"	"	Depressions.	Poor.	Light brown to grey.	Heavy.	"	"	Mottled grey, yellow, bluish-grey.	Heavy.	Compact.	"	Watchung.
Metamorphosed Triassic shale.	"	Level to sloping.	Good.	"	"	"	"	"	"	Somewhat compact.	"	Lehigh.

The Lakewood sand is nearly white, almost pure quartz sand, with some dark stain or mixture of organic matter in the upper two to four inches. Under cultivation this organic matter soon disappears, leaving a nearly white sand. Below depths ranging up to about two feet or more, the colour is slightly yellow and continues so to about three feet, where parent geological material essentially like that beneath the Sassafras sand is encountered. The following profile of the virgin Lakewood sand was studied in the field near Colliers Mills, located in the south-eastern part of the area.

Horizon.	*Depth.*
A_0	Leaf mould—very scant—many bare spots with no mould.
A_1	0″– 2″—grey sand—loose—acid.
A_2	2″–22″—light grey sand with yellowish cast in spots or streaks. The lower limit of this layer is sharply defined but irregular, varying from 12 to 22 inches below the surface. Scattered streaks of chocolate-brown sand ¼ to 1 inch in thickness, may occur at this lower limit—loose—acid.
B_1	22″–40″—reddish-brown sand, slightly loamy with occasional dark brown spots—loose—acid.
C_1	40″ + —The material becomes lighter in texture, contains some fine gravel and is yellowish to slightly reddish-brown in colour—loose—acid.

Topography—Level to rolling or dune-like.

Native Vegetation—Very poor scrubby pine, scattering of scrub and chestnut oak, undergrowth of huckleberry, timber is scattering and in places the surface is quite bare of vegetation.

In imperfectly drained areas within this belt there are dark-coloured soils, usually sands, the dark colour being due to the presence of organic matter, which has accumulated in these soils, and not in the well drained soils, because of their permanently wet condition. In the most extensive soils of this character there is found beneath the dark-coloured surface layer, which ranges up to somewhat more than a foot in thickness, a grey sand to a depth of about three feet. This overlies an indurated layer acting as a hardpan, which has a rusty-brown colour and consists of sand cemented with organic matter, with varying amounts of iron oxide. The parent geological material lies immediately beneath the three to six inch layer of hardpan. Such soils are classified in the St. John's Series. The north-western boundary of this belt lies approximately along the boundary between Ocean and Monmouth counties, where it enters the area south-westward near Hornerstown. Thence it runs southward to New Egypt and thence south-westward by Pointville.

The second belt is one in which loams and sandy loams, classified in the Sassafras and Collington series, predominate. It extends from the line described at the end of the preceding paragraph north-westward to a line roughly parallel to and from one to three miles north-west of the main line of the Pennsylvania railroad. Just as the south-eastern belt may be described as one in which grey soils prevail, this belt is one in which yellowish and brownish soils predominate. This second belt consists of two sub-belts, the division line running approximately along the middle in a north-easterly south-westerly direction. The south-eastern sub-belt is dominated by the soils classified in the Collington series (texturally, mainly sandy loam, or fine sandy loam and loam). These soils are characterised by a well-developed or mature profile, which is fundamentally identical in principle with the normal or mature profile found throughout the region. In uncultivated areas it consists of the following:

1. A thin, dark-brown layer, the dark colour being due to organic matter. This layer ranges up to about three or four inches in thickness. It is usually relatively sandy, but contains considerable fine material.

2. A yellowish-brown layer containing about the same proportion of fine material as the surface layer. It is usually loose in forested regions, but in fields cultivated for many years, it is apt to bake on drying unless it is well supplied with organic matter. On account of its sandy nature it bakes less than heavier soils.

3. A brown, strong yellow-brown, or rusty-brown layer with a considerably higher percentage of clay than is present in the layers above. It is usually friable, often breaks into small angular particles, half-an-inch or less in diameter at the top of the layer, becoming larger and less well defined downward. The top of this layer lies at a depth ranging in the various types from about fifteen to twenty-four inches, and the bottom lies at thirty to thirty-six inches deep. The lower part becomes greenish-yellow and finally passes into the greenish sandy clay of the parent material, the glauconitic sands.

The Sassafras soils, associated in this belt with the Collington soils as soils of subordinate importance because less extensive, have a profile essentially like that of the Collington soils, generally with lighter colours throughout and with a light-textured, sandy parent material instead of the greenish sandy clay underlying the Collington soils. The third layer (C horizon) of the Sassafras soils usually has a lower percentage of clay than the corresponding layer in the Collington soils, and the colour of this layer is apt to be a brighter brown, reddish brown, or yellowish-brown than the somewhat rusty-brown colour in the Collington soils.

The north-western half of the second belt is dominated by a soil classified as the Sassafras loam. Collington soils do not occur extensively in it, though considerable areas of Sassafras sandy loam are found.

The Sassafras loam occupies all the even upland watershed areas, while the sandy loam is found mainly on the slopes and along the hilly belts bordering on the valleys. These sandy loam soils are apparently due, at least in part, to their freshness or immaturity and imperfect development as soils and it will be remembered that the soils of the Sassafras series are underlain by rather sandy materials. The loam of the smooth uplands between the streams has weathered long enough to have developed a rather thick subsoil layer (B horizon), and the surface has decomposed to a loam. The sandy loam on the slopes is eroded continually, and lies lower topographically, so that a larger part of it is developed from the sandy layer normally underlying the loam, the materials of the three layers not having been exposed to the dynamic forces of soil formation for a sufficiently long period to complete decomposition.

A typical profile of an undisturbed Sassafras loam observed in the field two miles west of Jamesburg showed the following characteristics.

Horizon.	*Depth.*	
A_0	0″– 1½″	—Dark black leaf mould—acid.
A_1	1½″–6″	—Yellowish-brown friable loam containing considerable fine sand—acid.
A_2	6″–13″	—Reddish-yellow friable loam—acid.
B_1	13″–18″	—Reddish-yellow friable heavy loam—acid.
C_1	18″–25″	—Reddish-yellow friable heavy sandy loam—acid.
C_2	25″+	—Slightly reddish-brown heavy loose loamy sand to light sandy loam—acid.

Wherever found, the Collington soils contain greensand (glauconite) in varying quantities. These soils do not occur extensively north-east of Trenton or in the northern part of the district, except in a few small areas on the lowest slopes of the tributaries of the Raritan River and one or two areas along the Assanpink Creek. The topography of this district is, in general, too elevated for the exposure of the geologic beds from which typical Collington soils have been developed, as they lie beneath those from which the Sassafras loam and sandy loams of this sub-belt owe their origin. In this connection it should be stated that the Collington soils have been differentiated from the Sassafras soils almost wholly on the basis of the character of the parent geological formation to which they owe their origin, the Collington soils having been developed from beds containing a noticeable percentage of glauconite, while the beds giving rise to the soils of the Sassafras series do not contain this mineral.

The third belt of soils in central New Jersey is a belt of silty soils, and it occupies the north-west corner of the district. In this area the soils have been developed from material accumulated in the place it now occupies through the disintegration of consolidated materials (sandstones, shales, and dark coloured crystalline rocks), in contrast with the soils of the other two belts which have been developed from unconsolidated sands, silts, clays and glauconite beds accumulated by sedimentation in water, presumably sea water. In the southern part of this belt, a few miles north of Trenton the Lansdale soils have developed from fine-grained sandstones (argillite) and the profile is essentially like that of the Sassafras soils in all respects except (1) the surface texture, which is characterised by much less sand, and (2) the nature of the layer immediately beneath the B horizon (the heavy subsoil layer). In the Lansdale soils this layer (the upper C horizon) is intermediate in textural character between the Sassafras and Collington soils, usually heavier than that of the Sassafras and lighter than that of the Collington and it is relatively thin. Below this layer consolidated sandstone is reached at relatively shallow depths.

The Penn soils, occupying the greater part of this belt, have two marked characteristics through which they vary in their profile from the soils of the region as a whole. This regional profile, it will be recalled, is marked by a relatively light textured surface layer ranging up to more than a foot in thickness (leaving the thin leaf-mould layer in the timbered soil out of account) and a relatively heavy layer whose lower boundary lies about three feet deep, the colour being yellowish in the surface horizon and deeper yellow-brown to faint reddish-brown in the heavier horizon. The third horizon (that of the parent material or C horizon) is heavy or light in texture according to the nature of the parent material and varies also in other respects according to the particular characteristics of that material.

In the third belt as a whole the parent material varies considerably in character, but in the area of the Penn soils, and especially the Penn silt loam, it is relatively uniform and consists of dark-red shale. This shale decomposes slowly on account of its density and of the slowness with which water appears to penetrate it. The layer of disintegration is thin, so that the soil is necessarily shallow and the soil profile is not so well developed as in the Sassafras and Collington loams and sandy loams.

In addition to the slow weathering of the parent materials from which the Penn silt loam is developed, these rocks have a characteristic colour that differentiates them from the other rocks of the region and that colour is also resistant to weathering. This brings about a slow development of the colour profile of the soils formed from them, just as their slow disintegration, and to a certain extent their fine grain, results in a slow development of the texture profile of these soils. The colour of the parent material is still present at much shallower depths in the soil than in the majority of the soils developed from the unconsolidated materials lying to the south-east. The whole of the profile of the Penn soils is therefore predominantly red.

The following profile of the virgin Penn silt loam was observed and recorded near Franklin Park in the north central part of the New Jersey area.

Horizon.	*Depth.*	
A_0	½″– 1″	—Dark leaf mould.
A_1	1″ – 6″	—Dark brownish-red to Indian red, rather mellow silt loam—acid.
B_1	6″ –16½″	—Indian red more compacted silt loam—acid.
B_2	16½″–31½″	—Indian red—heavy silt loam—quite compact—acid.
C_1	31½″–46″+	—Indian red—heavy silt loam containing an abundance of small fragments of red shale.

Bed rock shale at 56″.

In the following paragraphs are given brief summaries of the characteristics of all the soil series identified and classified in the various soil belts of the central New Jersey area.

North-western Belt.—The types of the Penn series are derived from red sandstones and shales of Triassic age. They are characterised by Indian red or reddish-brown surface soils and brighter chocolate red or Indian-red subsoils. The colour of the soil is much the same as that of the parent rock. The Penn silt loam and Penn shale loam were the only soil classes found.

The soils of the Lansdale series are derived principally from dense greyish argillite, and greyish sandstone or shale. The surface soils are brown and overlie brownish-yellow subsoils. They occur as stony and gravelly loams and silt loams.

The Croton soils have greyish-brown or grey surface soils and are underlain by mottled yellowish and greyish subsoils overlying a very compact hardpan layer of reddish-brown or mottled rusty-brown clay. They are imperfectly drained and adjoin the Penn and Lansdale soils. Only the Croton silt loam occurs in the area.

The soils of the Montalto series owe their origin to the disintegration of dense, massive trap rock (diabase) of Triassic age. The surface soils are reddish-brown in colour and are underlain by reddish-yellow subsoils. Stony loam, gravelly loam and silt loam were classified.

The Watchung series are imperfectly drained soils derived from trap rock. The surface soils are light brown or grey, the subsoils are mottled-greyish, yellowish and bluish grey. The lower subsoil is often compact and impervious. The series occurs in this area only as silt loam.

The Lehigh soils have light brownish-grey or grey surface soils overlying mottled greyish, yellowish and bluish-grey or dove-coloured subsoils. They are derived from sandstones and shales that have been altered by the intrusion of great masses of molten trap rock, which apparently subjected them to great heat and compression. The Lehigh shale loam and Lehigh silt loam were classified.

Central and South-eastern Belts.—The Sassafras series is characterised by the brown colour of the surface soil and the reddish-yellow colour and friable character of the subsoil. In the heavier types the lower subsoil is lighter in texture and frequently contains gravel or sand or both. These are well drained soils and occur in a great range of textures.

The types of the Woodstown series have brown to light-brown surface soils and mottled grey and yellow or bluish-grey and yellow subsoils. These types, in the surface layers, are much like those of the Sassafras series, but in the subsoil they are more like the Elkton soils. The mottled subsoil indicates poor drainage. The loam and sandy loam were identified.

The Elkton series includes types having ash-grey to light-grey surface soils, overlying mottled yellowish-grey or bluish-grey subsoils. The mottling in the subsoil is due to imperfect drainage. At depths of about 30 to 40 inches the material is frequently lighter textured than it is above. The sandy loam, loam and silt loam occur in central New Jersey.

The types of the Portsmouth series are dark-grey to black in the surface layer, and light grey, grey, or whitish in the subsoil, which is mottled in the lower part with yellow, grey and bluish-grey. These soils have poor drainage. Only the sandy loam and loam were identified and classified.

The Collington series includes types with brown to dark reddish-brown surface soils and reddish-yellow or greenish-yellow subsoils. They contain greensand (glauconite) throughout the soil profile, usually more in the subsoil than in the surface layers. In many places the subsoil has a rather greasy feel and is of an olive green colour, the intensity of the colour depending on the quantity of glauconite present. These are well drained, well oxidised soils. In central New Jersey they are very extensive soils with a great textural range.

The types of the Colt's Neck series have brownish-red and reddish-brown surface soils overlying dark reddish-brown or reddish-brown subsoils with some yellowish material in the subsoil in places. Locally the lower subsoil contains partly decomposed greensand marl, which gives rise to greenish and reddish colours. These soils are not extensive, the loam and sandy loam being identified.

The sandy loam, loam and silt loam classes of the Shrewsbury series occur in central New Jersey. They have brownish-grey to grey surface soils overlying mottled greyish, yellowish, brownish, and greenish subsoils. Both soil and subsoil contain glauconite, and in places the quantity is sufficient to give the material a greenish colour. The Shrewsbury soils are imperfectly drained and occupy relatively low topographic positions.

The Keansburg series has a black surface horizon overlying mottled yellowish, greenish and reddish subsoils. It has poorer drainage than the Shrewsbury soils, occupies depressed topographic positions and contains much greensand marl in both surface and lower horizons. This series is represented in central New Jersey by the silt loam, loam and sandy loam.

The soils of the Keyport series have greyish-brown or brown surface horizons overlying lower horizons of yellowish and brownish colour, friable in the upper portion but stiff and plastic below. The lower layers consist of mottled yellowish, greyish and brownish, stiff, clayey material of the order of a clay-pan. These soils are not extensive in New Jersey, only the sandy loam and loam being identified.

The soils included in the Lakewood series are characterised by the nearly white or very light greyish surface horizons and friable or loose orange coloured lower layers. They are well drained and in many places excessively drained. Only the sand and fine sand occur in this area.

The Leon series comprises soils with light-grey or white surface layers overlying a layer of compact sandy material, coffee-brown in colour, below which the material becomes orange or light yellowish-brown to yellow in colour and less compact. The Leon soils occupy depressed areas of poor drainage. They are not extensive in central New Jersey, only the fine sand being identified.

The soils of the St. John's series also have a compact or hardpan-like sandy layer of essentially the same character as the layer in the Leon. Above the coffee-brown compact layer the surface soil is black, and below this the lower horizons consist of looser, lighter coloured material, usually light brownish, yellowish, or mottled greyish and yellowish. In places there is a lighter coloured layer between the dark surface section and the coffee-brown compact layer. The sand and fine sand occur in the area.

In Table IV is shown a key to the classification of the soil series of central New Jersey according to the New Jersey system.

XI. DESCRIPTION AND PHYSIOGRAPHY OF THE SOUTH-EAST ENGLAND AREA.

The south-east England area is made up of over one-half of Kent and a small portion of the eastern part of Sussex. It comprises an area of approximately 700 square miles, and is bounded by the English Channel on the south and east, the North Sea and River Thames on the north and on the west by East Longitude 0° 44′. The Isle of Thanet and most of the Isle of Sheppey are included within its borders. Ashford, Margate, Dover, Folkestone and Canterbury are the principal towns within the area, and London is situated less than fifty miles from the north-west border.

The physiographic divisions of the area are well marked and directly related to the geological formations. These regions therefore extend in a north-west south-east direction and parallel each other.

Most of the area is situated on the Chalk, which extends as the North Downs in a broad expanse north-westward from the sea at an elevation of rarely less than 500 feet, and in places as high as 800 feet, and descends frequently by an abrupt escarpment to the Gault Valley on the south-west. The Lower Greensand ridge consisting of the Folkestone, Sandgate and Hythe beds and the Atherfield clay, gives rise to a distinct upland mostly at an elevation of about 300 feet. It is in places quite narrow and descends with a scarped face abruptly to the Weald on the south-west. This relatively low level plain extends for many miles beyond the area. Romney Marsh, situated in the extreme south-eastern part of the district, is a large level reclaimed tidal area which provides a sharp contrast with the much higher Hythe beds and Chalk Downs to the north. Other large tidal areas occur in the valleys of the River Stour and the Swale. The Isle of Thanet is occupied mostly by the comparatively elevated Chalk and the London Clay occurs at a relatively high altitude in the north central part of the area and in the Isle of Sheppey.

The area is drained by numerous streams, flowing into the Swale and the various branches of the Great Stour. The River Rother drains the south-eastern part.

XII. THE GENERAL GEOLOGY OF SOUTH-EAST ENGLAND.

The geological formations of south-east England are for the most part younger than those found in central New Jersey. They consist of both consolidated and unconsolidated deposits, mostly the latter, and are of Cretaceous, Tertiary and Post-Tertiary age. Structurally the Cretaceous formations are the remnants of the eroded northern portion of the dome of the Weald, each successive bed dipping toward the north with scarp faces, sometimes somewhat abrupt, facing the south.

THE GEOLOGICAL FORMATIONS OF SOUTH-EAST ENGLAND.

Lower Cretaceous Rocks.—The Lower Cretaceous rocks are the oldest formations of the district and consist of the Tunbridge Wells Sand and Wadhurst Clay. Both these unconsolidated materials occur only in the extreme south-eastern part of the area and are not extensive. The Tunbridge Wells Sand is overlain successively by the Weald Clay; Atherfield Clay; the Hythe beds, which consist of alternating layers of sandy limestone (Kentish rag) and a loose or slightly cemented sand (Hassock), and the unconsolidated Sandgate and Folkestone beds. The thickness of all the formations shows considerable variation and they occur in relatively narrow parallel belts extending in a north-westerly south-easterly direction.

Upper Cretaceous Rocks.—The lowermost formation of the Upper Cretaceous rocks is the Gault, a stiff plastic calcareous clay variable in depth of colour. The Upper Greensand is not found in this district but when present elsewhere it overlies the Gault. The Chalk tops the Gault in the area under consideration and consists of three members known as the Lower, Middle and Upper Chalk. These all consist primarily of a relatively soft porous marine limestone but the lower division is soft, more greyish in colour, containing only a few flints and in its lower part consists of marl containing considerable glauconite. The Middle Chalk is white and harder and contains only a few flints. An abundance of flints arranged in stratified formation is found in the Upper Chalk the rock itself being white in colour and an extremely pure slightly hard limestone. The Chalk is the most extensive formation of the district.

Lower and Middle Eocene Beds.—These are all beds of unconsolidated materials ranging in texture from stiff plastic clays to loose sands, sometimes containing gravel. The Thanet beds are the lowest of the Lower Eocene, and consist of fine sands slightly greenish in colour. Successively following them are the Woolwich and Oldhaven beds which closely resemble each other except that the Woolwich contain sands and clays while the Oldhaven is made up of sands. Both are characterised by the presence of black rounded water-worn pebbles of flint. The pebbles are more abundant in the Oldhaven beds. All these formations except the Oldhaven occur quite extensively along the north-east border of the Chalk. The Oldhaven is only found in a few scattered places.

The Upper member of the Lower Eocene beds is the London Clay. This occurs extensively in the north-central and north-western parts of the area. Most of the Isle of Sheppey is underlain by this formation. It is a stiff plastic clay, acid in reaction and usually brownish in colour, giving rise to large areas of very poor agricultural lands.

The Middle Eocene is represented by the Lower Bagshot Sands which consist for the most part of coarse sands together with thin lenses of reddish, yellowish and bluish sandy clays. In this district they occur only in a very small area capping the London Clay in the Isle of Sheppey and are therefore relatively unimportant in soil formation.

Older Pliocene.—The Lenham beds are of older Pliocene age. They consist of reddish sands containing a high percentage of iron and occur in scattered areas of varying size on the North Downs near the escarpment of the Chalk. In many places this formation has filled in " pipes " in the Chalk to a depth of many feet. It is very important as a soil forming material wherever found.

Post-Tertiary Beds.—The Post-Tertiary beds have a very important bearing on soil formation in the district. The less recent formations include the Clay with Flints, Plateau Loam, Coombe Rock, Sand and Gravel Terraces and Brick-earth. The Clay with Flints and Plateau Loam occur only on the Chalk, the former as a reddish-yellow quite stiff plastic clay, containing an abundance of angular Flints while the latter is a reddish-yellow to brownish-yellow mellow loam. Both show great variations in thickness and occur in rather extensive areas. The Coombe Rock is a compact calcareous drift formed under cold climatic conditions. The Sand and Gravel and Brick-earth of the River Terraces are materials laid down by streams which previously flowed at levels now above the present rivers. They are unconsolidated materials brownish to reddish-brown in colour and occur most extensively in the valleys of the present streams in the northern half of the district.

The recent formations are found in the first bottoms (valley floors) of the larger streams such as the Great Stour, Little Stour, the Swale and River Rother. They consist of recent alluvial deposits from various sources. Romney Marsh also owes its origin to a similar type of alluvial material.

The classification of the Geological Formations of south-eastern England is shown in the table following.

TABLE V.

Classification of Geological Formations in South-eastern England.

SEDIMENTARY ROCKS.

POST-TERTIARY.

Recent—Alluvium.
Pleistocene.
  Brick-earth.
  Gravel and Sand.
  Coombe Rock.
  Plateau Loam. } On the Chalk.
  Clay-with-Flints. }

TERTIARY.

Older Pliocene.
  Lenham Beds.
Middle Eocene.
  Lower Bagshot Sands.
Lower Eocene.
  London Clay.
  Oldhaven Beds.
  Woolwich Beds.
  Thanet Beds.

SECONDARY.

Cretaceous.

Upper Cretaceous.
  Chalk.
  Gault.
Lower Cretaceous.
  Lower Greensand.
    (*d*) Folkestone Beds.
    (*c*) Sandgate Beds.
    (*b*) Hythe Beds.
    (*a*) Atherfield Clay.
  Wealden Series.
    (*c*) Weald Clay.
    (*b*) Tunbridge Wells Sand.
    (*a*) Wadhurst Clay.

NO IGNEOUS ROCKS OCCUR.

XIII. THE CLIMATE OF SOUTH-EAST ENGLAND.

The climate of the south-eastern England area is quite equable in contrast with the rather cold winters and moderately warm summers of the New Jersey area. January is usually the coldest month and the ground rarely freezes except for a short period and then only to a very shallow depth. July and August are the warmest months. The annual average rainfall ranges from about twenty inches in the vicinity of Romney Marsh to as high as about thirty to thirty-five inches on the relatively high areas of the Chalk. In the northern and eastern part of the region it is again relatively low or around twenty inches per annum. October is usually the wettest month but the annual rainfall is so evenly distributed throughout the year, that in spite of the low total annual fall, it is usually sufficient to mature crops. At intervals, however, there are periods of excessive rainfall and other periods of serious drought.

Table VI below, shows the mean monthly rainfall of several locations in the south-east England area.*

TABLE VI.

Month.	Tenterden.	Dungeness	Folkestone.	Margate.
	El 190 ft	El.21 ft	El.230 ft	El.51 ft.
	ins.	ins	ins.	ins.
January	2.15	1.90	2.25	1.66
February	1.97	1.60	2.03	1.38
March	2.14	1.76	2.17	1.59
April	1.62	1.36	1.66	1.35
May	1.57	1.30	1.68	1.58
June	1.91	1.56	1.99	1.75
July	2.09	1.81	2.10	1.98
August	2.29	1.98	2.39	1.93
September	2.14	2.05	2.37	1.97
October	3.49	3.52	4.03	2.92
November	3.02	2.75	3.25	2.41
December	3.11	2.77	3.21	2.28
Total	27.50	24.36	29.13	22.80

XIV. SOILS OF THE SOUTH-EAST ENGLAND AREA.

The soils of the south-east England area have been studied and classified in the field after the same system and manner as those of the New Jersey area. Here, as in New Jersey there is a very striking relation between the geology of the region and the soil types. Moreover there is the same more or less belted arrangement, extending like the geological formations, in a north-west south-east direction.

The most south-easterly belt occurs in the south-eastern part of the area in the Weald. This is exclusively an area of very heavy soils for the most part imperfectly drained. Fewer soil types are found in this district than in any other part of the area studied. There are two principal soils in the region differing primarily in their drainage which in turn affects the physical condition, profile and colour. They have been classified in this study as the Thorne Silty Clay Loam and the Hildenborough Silt Loam. In places the Hildenborough Silty Clay Loam and Silty Loam are found.

* From Air Ministry, Meteorological Office, 1924, pp. 226-227.

The Thorne Silty Clay Loam is a yellowish-brown to greyish-brown heavy silty clay loam mottled with greyish and dark reddish-brown colours, which at about eight to ten inches becomes a heavy plastic stiff, yellowish and greyish mottled clay, which passes at about thirty to thirty-six inches into the geological material, the Weald clay. The drainage of this type is poor and it is found in level to gently sloping topographic situations.

The Hildenborough soils are not as heavy in texture as the Thorne and have a more mellow surface layer which is more yellowish-brown in colour, unmottled and underlain at a greater depth by stiff, plastic clay. The drainage is fair and it occupies level to rolling positions. The Hildenborough soils can be used, when artificially drained, as arable land but the heavy texture, poor physical condition and inadequate natural drainage of the Thorne soils makes them better adapted to permanent pasture and forest growth.

The Wadhurst clay member of the Wealden Strata gives rise to soils of the Lamberhurst series. These soils have a greyish-brown, heavy stiff surface layer of silty clay loam or silt loam overlying a yellowish to greyish-brown stiff clay mottled with greyish-yellowish and brownish shades passing below into the geologic material of the Wadhurst clay. The Lamberhurst soils have fair to poor drainage and are found in nearly level to rolling topographic positions. These soils need under-drainage to improve the physical condition of both the surface and deeper layers. They occur only in the extreme south-western part of the area.

In the vicinity of Appledore in the south-western part of the area adjacent to Romney Marsh there occurs an outcrop of the Tunbridge Wells sand. This formation gives rise to the Pembury soils. Due to the nature of the geologic materials from which these soils are formed, they are more sandy and lighter in texture than most of the soils occurring in the Weald. The Pembury soils have a deep brown surface layer overlying a yellowish-brown to yellow layer which contains more sandy material than the layer above and is therefore lighter in texture. At about twenty-four to thirty inches (the B horizon) this soil becomes somewhat compact, due to the accumulation of physical and chemical materials carried by gravity and percolating waters from the eluvial horizons above. The geological material of the C horizon underlies this layer.

It should be here noted that only a relatively small portion of the entire Weald is included within the boundaries of the area discussed in this paper, but field examinations by the author outside the area now being discussed have shown that all the soils described above occur very extensively throughout the Weald. Soils derived from the Paludina Limestone (Bethersden Marble) have also been examined. Inspection has been made of several soils derived from the Tunbridge Wells sand ; this formation, owing to lithologic variations in the geological material, has given rise in other parts of the Weald to several soil series.

To the south-east of this belt of Pembury soils lies the Romney Marsh, a broad alluvial deposit, some of it reclaimed from the sea even before Roman times. No attempt has been made to classify the soils of this region, but preliminary studies by the author have shown them to consist of several types with variations in profile, texture and drainage, which no doubt accounts in a large measure for their great variation in agricultural value both for pasture and arable crops.

A third belt of soils occurs to the north-east adjacent and parallel to the Weald. These soils owe their origin to the lowest member of Lower Greensand formations, which is the Atherfield Clay. They occur only in a very narrow belt extending from north-west to south-east and are classified in the Charing and Westwell series. These soils closely resemble each other in all respects except that the Westwell soils occur in topographic depressions while the Charing series is found on hillsides with sloping or nearly level topography. The surface layers are heavy in texture and are brownish-grey in colour and overlie stiff yellowish plastic clay mottled with greyish, reddish-yellow and reddish-brown ; the lower layers often contain blackish concretions. These soils have fair to poor drainage and frequently receive considerable seepage water from the high levels of the Hythe beds. As they also frequently receive considerable wash from this adjoining formation, they are neutral or alkaline in reaction. In some places considerable Hythe material has been washed down over the sedentary soil derived from the Atherfield clay. Soils of this character have been classified in the Linton series and differ from the Charing and Westwell soils in having a lighter textured surface layer and containing varying amounts of glauconite. These soils also have better drainage and more friable surface layers.

The Hythe beds form the next belt of soils. In this area these beds are alternate layers of Hassock and Rag. They give rise to several soil series differing widely in the colour, texture and depth of the various soil horizons as well as in their topography and drainage. In many places the materials of the Hythe beds have been re-worked with materials from the nearby Folkestone and Sandgate beds. These conditions have given rise to a belt of very detailed soils which when classified after the New Jersey method according to their (1) geological origin, (2) mode of formation, (3) topographic position, (4) drainage, and (5) profile, show the following characteristics.

CHART SERIES

(1) Hythe Beds, (2) Sedentary, (3) Good to excessive (on high elevations), (4) Level to rolling, (5) Brown to reddish-brown surface layers containing glauconite overlying reddish-brown with greenish-brown material, below which the rock is encountered

Bed rock at any depth, usually shallow on the shoulders of hills and top slopes, less shallow on slopes, deep in flats and depressions

Medium organic matter in surface layers—neutral to alkaline in surface layers, highly calcareous in lower layers.

Notes.—There are five phases in the Chart Series :

(1) Shallow phase	1 ft. to bed rock.
(2) Medium phase	1 ft. to 2 ft. bed rock.
(3) Deep phase	2 ft. or more to bed rock.
(4) Rag phase	C1 horizon—Rag.
(5) Hassock phase	C1 horizon—Hassock.

The depth of the bed rock in the Chart series is exceedingly variable. The author has observed it in the field from six inches below the surface to three feet or more. It is usually less than three feet.

AMBERFIELD SERIES.

(1) Hythe Beds, with some slight Folkestone and Sandgate material. (2) Mostly sedentary, some re-working (3) Flat to gently sloping. (4) Good. (5) Brown surface layer to depth of twelve to eighteen inches containing some glauconite overlying to a depth of twenty-four to thirty inches, reddish to orange, heavier textured material, containing fragments of rag and considerable glauconite and underlain by orange coloured heavy material, mottled with yellowish, reddish and greenish shades.

Surface layers lighter in texture than lower layers, lower layers compact. Bed rock usually at about two and a half to three feet.

Surface layers—neutral to alkaline, deeper layers alkaline. Fair content of organic matter in surface layers.

TABLE VII.

Key to the Classification of the Soil Series of South-East England.

Geological Material.	Mode of Formation.	Topographic Position.	Drainage.	PROFILE.								
				UPPER HORIZONS.				LOWER HORIZONS.				
				Colour.	Texture.	Consistency.	Reaction.	Colour.	Texture.	Consistency.	Reaction.	Series Name.
Weald clay.	Sedentary.	Level to gently sloping.	Poor.	Yellowish-brown to greyish-brown mottled greyish and dark reddish-brown.	Heavy.	Plastic.	Acid.	Yellowish and greyish mottled.	Very heavy.	Very plastic	Acid.	Thorne.
Weald clay.	,,	Level to rolling.	Fair.	Yellowish-brown.	,,	Slightly mellow.	,,	Yellowish and greyish mottled.	Heavy.	,,	,,	Hildenborough.
Wadhurst clay.	,,	,,	Fair to poor.	Greyish-brown.	,,	Stiff.	,,	Yellowish to greyish-brown, grey, yellow and brown mottled.	,,	Very stiff.	,,	Lamberhurst.
Tunbridge Wells sand.	,,	Rolling to level.	Good	Brown.	Light.	Loose	,,	Yellowish-brown to yellow	Slightly heavy.	Somewhat compact.	,,	Pembury.
Atherfield clay.	,,	Depressed.	Fair to poor.	Brownish-grey.	Heavy.	Stiff	Alkaline.	Yellowish-grey, reddish-yellow, reddish-brown mottlings, black concretions	Very heavy.	Stiff plastic impervious	Alkaline	Westwell.
Atherfield clay.	,,	On hillsides sloping to level.	Fair to poor —seepage water	,,	,,	,,	,,	,,	,,	,,	,,	Charing.
Hythe material overlying Atherfield clay.	Colluvial.	Gently sloping to level.	Fair to good	Dark-brown greyish and greenish.	Fairly heavy.	Friable. mellow.	,,	Yellowish-brown, grey-green mottling.	,,	Stiff plastic.	,,	Linton.
Hythe beds.	Sedentary.	Level to rolling.	Good to excessive	Brown to reddish-brown greenish.	,,	Friable	Neutral to alkaline.	Reddish-brown, greenish.	Fairly heavy. Bed rock may occur at any depth.	Friable	,,	Chart.
Hythe beds small amount Folkestone and Sandgate material.	Mostly sedentary re-worked.	Gently sloping to flat.	Good.	Brown-greenish	,,	,,	,,	Orange, red, yellow, green mottling.	Heavy.	Compact	Alkaline.	Amberfield.
Hythe beds.	Sedentary.	Flat highland	Good to slightly imperfect.	Reddish-brown to brown	,,	,,	Alkaline	Red, red, yellowish-brown and green mottlings.	Very heavy.	Stiff plastic.	,,	Knowle.
Hythe and Folkestone beds.	Re-worked.	Rolling to level.	Good to excessive.	Brown.	Light.	Loose	Acid.	Brown to rusty-brown.	Light.	Loose.	Neutral.	Ulcombe.
Sandgate beds.	Sedentary.	Sloping to level.	Fair to good.	Green.	Heavy.	Plastic.	Neutral to acid.	Green.	Heavy.	Very plastic	,,	Stone Hill.
Folkestone over Sandgate material.	Re-worked.	Sloping.	Good.	Brown.	Light.	Open.	Acid.	Yellowish and greenish-brown, yellow, green and reddish-brown mottlings.	Fairly heavy.	Fairly plastic.	Acid.	Sevington.
Sandgate over Folkestone material.	,,	Gently sloping to level.	Good to excessive.	Brown.	Fairly heavy.	Mellow.	,,	Yellowish-brown.	Fairly light.	Loose.	,,	Leacon.
Gault.	Sedentary.	Rolling to level.	Imperfect to poor.	Brown with green rusty-brown and grey mottling.	Very heavy.	Plastic.	,,	Greenish-grey, grey and yellow mottlings.	Very heavy.	Stiff plastic.	,,	Broadway.
Folkestone beds.	,,	Sloping to level	Excessive.	Black 6" to 8", grey 24".	Very light.	Loose	,,	Brown.	Light.	Cemented impervious.	,,	Hothfield.
Folkestone with slight content Sandgate.	Re-worked.	,,	Good to excessive.	Brown to yellowish-brown.	Light.	,,	,,	Yellowish-brown to yellow. Micaceous.	,,	Loose.	,,	Surrenden.

Other soil series found on the Hythe beds were identified in the field and classified as the Knowle and the Ulcombe. The Knowle has reddish-brown to brownish surface layers overlying brick red to Indian red plastic clay mottled subsoils. The Ulcombe the soils of which owe their origin to the re-working of Folkestone and Hythe materials has a very open profile and consists of deep soils containing considerable sand derived from the Folkestone beds.

From the foregoing descriptions of the various soil series found on the Hythe beds, it is quite evident that this belt of soils is one showing considerable variation in texture and colour as well as topography. Generally, however, the belt is much lighter in texture than those derived from the Weald clay to the south-west. It is upon the soils of the Hythe beds that many of the orchards of Kent are situated.

The Sandgate beds lie north-east of the Hythe beds and roughly parallel to them. They are in turn adjoined on the north-east by the Folkestone beds. There has been much re-working of materials of these two formations, but on the whole the original materials of the Sandgate beds being mixed clays and glauconitic sands have given rise to a belt of soils lighter in texture than those found on the Hythe beds, while the Folkestone beds, made up mostly of fine sands has given rise to a belt of very light textured sandy soils composed for the most part of fine sand.

The soils derived from the Sandgate beds have been classified in the Stone Hill series when they are of sedentary origin. These soils are quite plastic in both surface and deeper horizons and very green in colour. The drainage is good to fair and they usually occupy gentle slopes. They represent soils derived from the outcrops of clayey Sandgate beds, uncontaminated with other materials.

In many places soils are found consisting of Folkestone materials re-worked over the Sandgate beds. Such soils are classified in the Sevington series. They are easily recognised by the brown to slightly yellowish-brown surface layers underlain by mottled yellowish, slightly reddish-brown and greenish layers at about twenty-four inches. The mottling is due to the geologic materials of the Sandgate beds and not to poor drainage.

There are three important soil series derived from materials made up wholly or chiefly of the Folkestone beds. They consist primarily of fine sandy materials which give rise to very light sandy soils which in Kent are frequently " Common Land " or " Heath Land." These soil series differ principally in the colour of the upper and lower horizons.

The Hothfield series is perhaps in Kent the most extensive of these. It is derived entirely from Folkestone materials. The upper horizon of six inches to eight inches is black fine sand passing at about six inches to eight inches into grey fine sand which becomes a brown and cemented hardpan layer (B horizon) at about twenty-four inches. Below the hardpan layer, the soil becomes yellowish-brown in colour. The Hothfield soils are without a doubt true podsols.

In some places the Folkestone beds have been slightly re-worked with Sandgate materials for the most part of sandy texture. Such conditions give rise to two important soil series classified as the Surrenden and Potters. Both series have yellowish-brown and brownish surface horizons six inches to eight inches deep, but differ in that the Surrenden soils have yellowish-brown to reddish-brown deeper horizons and the Potters are pale yellow in colour in the deeper layers.

All of these three series are characteristically soils having a very sandy profile, excessive drainage and varying degrees of podsolisation.

In some situations (e.g., about two miles south-east of Charing) the Sandgate Beds have been re-worked over the Folkestone giving rise to a series of soils having brown mellow surface layers which at about twenty-four inches becomes more yellow and sandy and contain slight amounts of glauconite. These soils have good to excessive drainage due to the open and sandy nature of the deeper layers derived from Folkestone materials. They have been classified in the Leacon series.

The Gault lies to the north-east of the Folkestone beds and gives rise to a belt of very stiff plastic soils which stand out in bold contrast to the sandy soils derived from the Folkestone and Sandgate beds to the south-west, and those derived from the Chalk to the north-east. Due to the topographic position between the relatively high Greensand beds and Chalk they occupy a relatively low elevation although the land surface is in many places quite rolling. The soils derived residually from the Gault clay have imperfect drainage and have been classified in the Broadway series. They consist texturally chiefly of very stiff plastic materials both in the surface and subsoils. The surface soil is brown with greenish shades and mottlings of dark rusty-brown and greyish-green to a depth of nine or ten inches, overlying greenish-grey stiff plastic heavy clay loam to clay subsoils, slightly mottled with lighter green, greyish-green and some yellowish-green. There is little change in colour or texture to a depth of forty inches or more. Whitish concretions or nodules of phosphate of lime are a common occurrence throughout the profile of these soils. Due to the heavy texture, undesirable physical condition and poor drainage of these soils, practically none of them are arable land but they are extensively used for pasture, some of which is quite poor. The silt loam, silty clay loam and clay loam were identified.

In the field examinations of the soils of the Gault, it was recognised that in some areas adjacent to the Greensand and Chalk there are narrow belts of soils consisting of downwash material from the Greensand and Chalk overlying and adjacent to the Gault. These areas were not of sufficient size in this part of Kent, however, to warrant their classification, but it is recognised that they give rise to types of soils superior to those of the Broadway series. They also no doubt occur more extensively elsewhere in the southern part of England and should there be classified in separate soil series.

The soils of the Chalk are the most extensive of the area and perhaps the most interesting and difficult to classify. This is due to the fact that there has been considerable re-working of materials deposited by water over portions of the Chalk formation. These waterlaid materials are classified geologically as the Lenham beds (Older Pliocene in age) and the slightly younger Post-Tertiary Clay-with-Flints and Plateau Loam. The soils on the Chalk occur in a north-easterly belt many miles wide and lying several hundred feet above sea level with rather sharp escarpments on the south-west. In Kent the Chalk formation is a portion of the North Downs. The Chalk also underlies the greater part of the Isle of Thanet.

The soils derived from sedentary Chalk materials are classified in the Downland series. They are very shallow soils in which the bed rock Chalk is encountered at a depth of about one foot or less. The surface layers are light greyish-brown in colour usually containing fragments of chalk overlying slightly yellowish material which passes directly into the bed rock. In places where the soil is very shallow the yellowish layer is entirely absent. The Downland soils are usually fairly heavy in texture, consisting of loams and silty loams and they occur on slopes and in relatively level situations. Generally the more level the topography, the greater the depth to bed rock. Their drainage is excessive and they suffer greatly from drought. The Downland soils are

TABLE VII (Continued).

Key to the Classification of the Soil Series of South-East England.

Geological Material.	Mode of Formation.	Topographic Position.	Drainage.	PROFILE.								
				UPPER HORIZONS.				LOWER HORIZONS.				
				Colour.	Texture.	Consistency.	Reaction.	Colour.	Texture.	Consistency	Reaction.	Series Name.
Folkestone with slight content Sandgate.	Re-worked.	Rolling to level.	Excessive.	Brown to yellowish-brown.	Light.	Loose	Acid.	Yellow	Light.	Loose.	Acid.	Potters.
Chalk.	Sedentary.	Sloping to steep.	,,	Greyish-brown.	Fairly heavy.	Loose chalk fragments	Alkaline.	Bed rock chalk usually at about eight to twelve inches.				Downland.
Chalk.	Colluvial.	Level.	Good.	,,	,,	Mellow.	,,	Yellowish and greyish-brown	Heavy.	Mellow.	Alkaline.	Sidelands.
Clay with flints.	Sedentary.	Level to undulating.	Fair to good.	Brown.	Heavy—flints.	Fairly mellow.	Neutral to acid.	Reddish to yellowish-brown, red mottling. Chalk may occur at any depth.	Very heavy.	Stiff.	Variable.	Rattle
Plateau loam.	,,	Level.	Good.	,,	Heavy.	,,	,,	Reddish-brown	Very heavy.	Mellow.	,,	Sheldwich.
Plateau loam over Clay with flints.	Re-worked.	,,	Fair.	,,	,,	,,	,,	Yellowish-brown, grey, red mottling	Quite heavy.	Stiff plastic.	,,	Selstead.
Lenham sand over Plateau loam.	,,	,,	Good.	Red.	Fairly light	Loose.	,,	Yellow to yellowish-brown	Very heavy.	Mellow.	Neutral to acid.	Maxted.
Plateau loam over Lenham sand.	,,	High position.	,,	Reddish-brown.	Fairly heavy.	,,	,,	Reddish-brown, greenish.	Light.	Loose.	,,	Elmsted.
Flints and Tertiary over Chalk.	,,	Level	,,	Reddish-brown.	Fairly heavy Flints and Tertiary gravel	Mellow	Alkaline.	Yellowish-brown. Bed rock chalk usually at about two feet	Heavy.	Mellow.	Very Alkaline.	Nicholas.
Thanet beds.	Sedentary.	Level to undulating.	Good to excessive.	Brown.	Light.	Loose	Neutral.	Yellowish-brown	Slightly heavy.	Loose.	Neutral.	Hall
London clay	,,	Level	Fair to imperfect.	Yellowish-brown.	Very heavy.	Somewhat plastic.	Acid.	Yellow with grey and orange mottlings.	Very heavy.	Stiff plastic.	Acid	Blean.
Bagshot sand over London clay.	Re-worked.	Sloping to steep.	Good.	Brown to reddish-brown.	Heavy.	Slightly sticky.	,,	Yellowish-brown.	Very heavy.	Slightly plastic.	,,	Minster.
Brick-earth.	Terrace.	Gently sloping to level.	,,	Brown.	Fairly heavy.	Very mellow.	Neutral to acid.	Reddish-brown.	Fairly heavy.	Very mellow.	Neutral to acid.	Wye.
Brick-earth (mostly) and Thanet sand.	,,	,,	,,	,,	,,	,,	,,	Reddish-brown, slightly yellow.	,,	,,	,,	Faversham.
Valley gravels	,,	,,	,,	Brown, slightly grey.	,,	Mellow.	,,	Reddish-brown	Light.	Loose gravelly.	,,	Willesborough.
Folkestone with some Sandgate and Hythe.	,,	,,	,,	Reddish-brown.	,,	,,	Slightly alkaline to acid.	Light reddish-brown.	Fairly light.	Loose.	Slightly alkaline to acid.	Burton.
Gault (mostly) some Lower Greensands and chalk	,,	,,	,,	Greyish-brown.	,,	,,	Alkaline.	Reddish-yellow.	Fairly heavy.	Stiff gravelly.	Alkaline.	Eastwell.
Flints and Tertiary over London clay.	,,	,,	Fair to good.	Brown.	Heavy—flints and gravel.	,,	Acid.	Yellow, grey mottling.	Very heavy.	Stiff.	Acid.	Upstreet.
Brick-earth (mostly) some Thanet sand, Woolwich and Oldhaven.	,,	,,	Good.	,,	,,	,,	,,	Yellowish-brown.	Heavy.	Mellow.	,,	Sturry.
Sandgate and Hythe.	,,	,,	,,	Greenish, reddish-brown.	Fairly light.	Loose.	Alkaline.	Reddish-yellow, red-green mottling.	Fairly heavy.	Slightly plastic.	Alkaline.	Highworth.

essentially utilised for pasture, and due to their shallow depth and excessive drainage, no attempt should be made to bring these soils into cultivation.

In many places considerable colluvial Chalk material has accumulated over the bed rock giving rise to a deeper soil than the Downland. These soils are classified in the Sidelands series. They consist of a greyish-brown surface layer ten to twelve inches deep overlying a heavier slightly more yellowish to greyish-brown layer which directly overlies the Chalk. Bed rock is encountered at two feet or more. The Sidelands soils consist mainly of silty loams and when of sufficient depth produce fair pasturage and some arable crops. Their agricultural value depends entirely upon the depth of the soil above the bed rock. They have good drainage, usually level topography and a mellow consistency.

Considerable areas of the Chalk have been covered with the Clay-with-Flints. This geologic condition gives rise to a heavy brown mellow surface soil overlying a reddish-brown to yellowish mellow horizon which is underlain at about twenty-four to thirty inches by a heavy horizon of yellowish-brown clay loam mottled with red. This soil usually contains an abundance of flints on the surface and is found typically on undulating topography. It occurs only in classes of heavy texture, such as loam, silty loam and silt loam. These soils have been classified in the Rattle series. In many places the Clay-with-Flints occurs only as a thin veneer over the Chalk, in other places it is very deep and therefore gives rise to deep or shallow phases of the Rattle soils. When the bed rock Chalk occurs within one foot, such soils are classified as shallow phases of the Rattle series while depths of soil from one to two feet and two to three feet are called intermediate and deep phases respectively of the same series.

The agricultural value of the Rattle soils for arable crops depends upon their depth to the chalk. When of sufficient depth (two and a half feet to three feet or more) good results are obtained but the intermediate and shallow phases are best utilised for pasture.

One of the best arable soils of Kent occurs on the Chalk and is found where the drift consists entirely of Plateau Loam. These soils have been classified in the Sheldwich series, and are usually excellent loams. The surface horizon is a brown to slightly greyish-brown slightly podsolised mellow loam containing considerable fine and very fine sand which at about six inches becomes more yellow in colour and heavier in texture to a depth of three feet or more. Due to their mellow consistency, excellent drainage and desirable physical qualities, the Sheldwich soils are excellent for arable crops.

In many places on the Chalk the Clay-with-Flints and Plateau Loam have been re-worked giving rise to a brown mellow loam, containing some flints, which at about twelve inches becomes slightly lighter in colour and is underlain at about twenty inches by a heavy horizon of yellowish-brown silty clay loam continuing to a depth of forty inches or more. At this depth there are slight mottlings of greyish, reddish and yellowish shades. Due to the heavy subsoil the drainage is only fair. Such soils are classified in the Selstead series. They are closely associated with the Rattle and Sheldwich soils, but differ from them in that the surface horizons are more mellow than those of the Rattle series and the deeper horizons are heavier, stiffer and more plastic than those of the Sheldwich soils. The Selstead soils can be utilised effectively as arable land.

Along the southern escarpment of the Chalk and extending in places for a distance of as much as five miles to the north-east of the escarpment, the Lenham Sand is found overlying the Chalk and occurring in scattered areas, sometimes of considerable size. These reddish sands are usually found associated with the Plateau Loam, Clay-with-Flints, and Chalk, with which materials they have been re-worked in varying amounts.

The soils formed in this manner have been grouped into two series depending upon the relative proportions of the various materials of which they consist, because all their properties have been radically influenced by this factor. The first of these soils is classified as the Maxted series and consists of Lenham sand material overlying Plateau Loam. This geological condition gives rise to a soil having a reddish coloured surface layer containing considerable sand which at ten to twelve inches becomes slightly more yellowish in colour and contains very sandy streaks of reddish colour. These horizons are derived from Lenham Sand material. At about twenty-four inches the profile becomes very heavy in texture containing very little sandy material and is yellowish to yellowish-brown in colour. This lower horizon owes its origin largely to the Plateau Loam. The Maxted soils have good drainage and level topography and should be excellent soils for arable crops.

The Elmsted soils consist of a mixture of Plateau Loam over Lenham Sand, the whole underlain by the Chalk. In places the Chalk is encountered at a relatively shallow depth, in other places it is many feet below the surface. These soils also contain considerable glauconite which gives them a greenish colour throughout the profile. The surface soils, however, are reddish-brown in colour, somewhat sandy in texture, and overlie at about ten to twelve inches more heavy and plastic horizons to a depth of about thirty to thirty-six inches where the profile becomes more sandy and reddish, indicating Lenham Sand material in this lower horizon. They occupy level, high, topographic positions and have good drainage.

Much of the Isle of Thanet is underlain by the Chalk which in turn most everywhere is overlain by a loamy material unclassified for the most part on the geological drift map. This overlying material which does not appear to bear any relation to the Chalk gives rise to a large area of excellent brown mellow loam utilised extensively in the production of potatoes, corn, lucerne and roots. It frequently contains flints and some Tertiary material. Soils of this character are classified in the Nicholas series. They consist of reddish-brown mellow surface horizons eight to ten inches deep, containing some flints and Tertiary gravel with rounded pebbles of flint underlain by slightly heavier textural layers yellowish-brown in colour. Bed rock Chalk is frequently encountered at about a depth of two feet although in some places the Chalk is found closer to the surface and in others at a greater depth. Under such conditions the Nicholas soils are classified as shallow phase, less than one foot to the Chalk, intermediate phase one to two feet to the Chalk and deep phase two or more to the Chalk. The agricultural value of the Nicholas soils is of course largely dependent upon the depth of the soil above the bed rock Chalk.

The Thanet beds occur north and north-east of the Chalk and are sandy. They occur most extensively west of Sandwich, east and west of Canterbury and near Faversham and Sittingbourne. Soils derived exclusively from the Thanet beds are classified in the Hall series. They are sandy in nature in the upper horizon and brown in colour to the depth of six to eight inches where the texture becomes more sandy to a depth of about twenty-four inches. The deeper horizons at about thirty inches are heavier in texture than those above. The Hall soils have excellent drainage and in places it is somewhat excessive. They are soils having a very open profile and give rise to a belt of excellent arable lands.

The Woolwich and Oldhaven beds overlie the Thanet Sands but have a very limited occurrence in the area studied. They are most extensive, about four miles north-west of Sandwich, near Herne, at Hernhill, and north-east of Sittingbourne. The Oldhaven beds contain large quantities of black rounded pebbles of flint while the Woolwich beds are made up mostly of sand with only a small amount of pebbles and clays. Due to the limited extent of these formations, no soil series were established for their derivatives but the following profile was observed one-half mile north of Wingham in the Woolwich beds.

Horizon. *Depth.*

0′ –2′ —Drab to grey coloured sandy loam to loamy sand becoming very gravelly at about two feet. The gravel is of rounded black flints.

2′ –2½′—Lighter coloured grey sandy loam to loamy sand.

2½′–4½′—Mottled reddish-yellow, greenish and yellowish sandy loam.

5′ or more—Light yellowish fine sand containing an abundance of glauconite and bands of reddish coloured sands which decrease with depth and finally disappear.

Overlying the Oldhaven beds is the London Clay. This formation occurs very extensively north of Canterbury and in the Isle of Sheppey. It gives rise to a very stiff plastic area of soils which are classified in the Blean series. Some variation in the surface texture of these soils was found in the field. In places they are very heavy and plastic and are a clay loam in texture, elsewhere the surface soils are silty clay loam and silt loam. The Blean soils have light yellowish-brown surface layers very heavy in texture and quite plastic; these are underlain at six to eight inches by heavy textured material, yellow in colour and mottled with grey and orange. Below twenty-four inches the layers are less grey in colour and more yellowish and contain some brownish shades. The entire profile of the Blean soils is quite compact and their cultivation greatly retarded by the heavy texture and undesirable physical condition of the surface layers. Much of the Blean soils, especially north-west of Canterbury, is in forest.

The Bagshot Sand tops the London Clay, but is found only in a very small area in the extreme north-eastern part of the area in the Isle of Sheppey. Here the geological material from the Bagshot Beds seems to be re-worked with the London Clay giving rise to a soil having a brown heavy surface horizon containing considerable coarse and medium sand and an abundance of rounded gravel which at about eight to ten inches is underlain by yellowish-brown mellow horizons containing more sandy material. At about twenty-four inches a very heavy textured layer is encountered, yellowish-brown in colour, containing a very high percentage of clay. These soils have been classified in the Minster series. In places there is considerable rounded gravel in the surface horizons and such areas are classified as gravelly types of the Minster series.

Post-Tertiary deposits classified as Gravel and Sand and Brick-earth have played a very important part in the formation of some of the soils in Kent. These deposits laid down by streams at a flow level now much higher than the present river beds, give rise for the most part to a group of excellent agricultural lands. Such soils occur most extensively in the valleys of the Great Stour, Little Stour and various tributaries of the Swale. Other rather extensive tracts occur near Deal and Blean. Due to the great assortment of geological materials involved, a great variety of soils has been formed in this manner, each having distinct profile characteristics, the nature of which is dependent largely upon the remnants of geological materials present. The horizons of the various profiles therefore differ in colour, texture, consistency, chemical reaction and sometimes in structure, and owing to these diverse characteristics their economic value for agriculture is also quite variable. This is largely due to the effect of the nature of the profile on the moisture relationships and the texture and consistency of the surface soil on cultivation. The recognition of the proportions and position in the soil profile of the various geological materials present is very important in the classification of this soil group which very strongly emphasises the importance of geology in the formation of the soils of the region, as a whole.

Perhaps the most extensive and important soils of this nature have been classified in the Wye series. The surface soils are brown, overlying light reddish-yellow horizons. Silt is abundant throughout the whole profile and some small amounts of sand mostly fine, and very fine are present. The profile throughout is very mellow, drainage is good and the Wye soils occur in flat to gently sloping positions. The geological material from which these soils are formed is mostly Brick-earth. They are excellent arable lands, perhaps the best arable land in Kent.

The Faversham soils also owe their origin mostly to the Brick-earth, but also contain varying quantities of fine sand from the Thanet Beds. They therefore differ from the Wye soils in having more sandy deeper horizons at about thirty to thirty-six inches. The surface layers are brown in colour to a depth of eight to ten inches becoming reddish-brown below. The whole profile is very mellow in consistency.

In the Great Stour valley are soils formed chiefly from materials from the Folkestone Beds, together with some from the Sandgate and Hythe Beds. They are reddish-brown on the surface overlying light reddish-brown horizons to a depth of about three feet. Glauconite is present throughout the profile. They have a sandy rather open profile and are classified in the Burton series. Elsewhere in the valley of the Great Stour, as at Kennington, stratified soils occur. The stratified soil is underlain by geologic material also stratified and frequently many feet in thickness. The lower horizons of the soil are gravelly consisting mostly of flints from the Chalk. Such soils have a very open profile and sometimes excessive drainage. The surface horizons are brown overlying reddish-brown lower horizons more sandy in texture. Such soils have been classified in the Willesborough series.

The Eastwell soils belong to the same group and consist mostly of Gault material together with some sands from the Lower Greensands, together with gravel and colluvial Chalk. The surface layers are greyish-brown overlying reddish-yellow heavy textured clay containing some gravel in the upper part. They have good drainage and level topography.

Other less extensive terrace soils have been classified as follows: Sturry series consisting mostly of Brick-earth with considerable Thanet Sand, Woolwich and Oldhaven materials, the Upstreet series occurring near Upstreet where the London Clay is covered with a thin re-worked veneer of flints and Tertiary sands and gravel, usually very thin but giving rise to a soil much superior to that derived from the London Clay, and the Highworth series derived mainly from re-worked Sandgate and Hythe materials and containing considerable glauconite.

No detailed attempt was made in the field observations upon which this paper is based to classify in detail the alluvial soils of the districts which consist of the first bottoms (valley floors) of the various streams and the rather extensive levels along the Swale, Great Stour, and in Romney Marsh and between Deal and Sandwich. The few field observations made, however, indicated, as is to be expected by the great variety of materials contributing to the formation of these lands and their alluvial origin, that they have quite heterogeneous profiles. These variations no doubt account for the well known differences in their agricultural value, either for arable crops or pasturage.

Table VII shows a key to the classification of the soil series of south-east England.

XV. DISCUSSION.

The Influence of Geology and Climate on Soil Types in South-East England and Central New Jersey.

1. The Dynamics of Soil Formation.

The Russian ideas of soil formation and classification have received much attention during the past decade and justly so, for they are the result of many years of research by a comparatively large personnel of very able scientists under the leadership of Glinka. The Russian school points out that soil formation is brought about by the action of climatic, biotic and edaphic factors upon given geologic materials. Soil formers play a very important part in this process and are divided into *active* and *passive* factors. Climate (rainfall, temperature and humidity) and the living element or Biosphere constitute the active factors; while parent material, topographic relief and time are the passive factors. It is further set forth that active factors have a direct effect upon soil formation while the action of passive factors is indirect. While this explanation is no doubt scientifically entirely satisfactory when applied to climatic and geologic conditions in Russia, the author does not feel that the same active and passive soil formers related above have the same direct and indirect influence on soil formation in other parts of the world. As soil science is rapidly becoming of international importance largely through the efforts of the International Society of Soil Science, it is highly desirable if possible to arrive upon some explanation of soil formation and at some system of classification which will find satisfactory application throughout the world. With this thought in mind, the following discussion is offered with special reference to soil conditions observed in south-east England and central New Jersey.

It is generally recognised that everywhere in the world soil formation and development are dependent upon four factors, viz. :—(1) The nature of the original soil forming materials. (2) Climatic factors such as rainfall and temperature. (3) The nature of the native vegetation. (4) Time. These are all very closely related and often dependent upon each other. The end product of the processes and materials is the soil, the morphology of which finds expression in the soil profile. In the development of the soils of any region, one or more of those factors is *dominant* and others *recessive*. Some examples of this principle deserve recognition here. In the western part of the United States, that is, in the States of North Dakota, South Dakota and Iowa, the author has examined and found great differences in the nature of the soil profiles of large areas of soils derived from geological materials having a common origin and mode of formation, namely: glacial and loessial deposits, materials laid down by the various agencies of glaciation and the wind. Further study revealed that these profile changes followed the climatic zones, and are therefore due to the *dominance* of the climate in the formation of the soils of the region in question. In other parts of the central United States, large areas of soils containing surface horizons rich in organic matter adjoin areas of soils containing much less organic matter in the surface layers; all these soils are derived from common geologic materials subject to quite uniform climatic conditions. Obviously factors other than geology and climate have here been *dominant* in their formation. A study of the other natural conditions of the region shows clearly that the soils containing a high percentage of organic matter in the surface horizons have been developed under a native vegetation of grass while those containing much less organic matter have matured under a forest growth. In this portion of the world therefore, the native vegetation has been *dominant* in the formation of the soils, and this is revealed in a morphologic examination of the soil profiles.

In England and New Jersey, of course, all these dynamic forces of soil formation have been, and are at work. In studying the results of the examination and classification of the soils of these regions, the most striking feature of the morphologic characteristics of the profiles and classification of the soils is the close relationship between them and the geologic formations. It is at once apparent that the upland soils have been formed under a native forest vegetation from the fact that the surface horizons are relatively deficient in organic matter. Climatic effects are reflected in the degree of podsolisation shown in the soil profiles but the nature of the geological materials has also radically influenced the podsolic process. It is therefore concluded that in the formation of the soils of south-east England and central New Jersey the geologic factor is *dominant* and the climatic, native-vegetation and time factors *recessive*. These conclusions differ radically from that maintained by the Russian school of soil scientists and especially Glinka (1928), who has expressed the opinion that the mature soil developed under uniform climatic and vegetative conditions, will be the same regardless of the nature of the parent geologic material. The author is unable to concur with this view as applied to south-eastern England and central New Jersey and has made a special study of the influence of geology and climate upon the classification and nature of the soils of these two regions, the results of which are set forth in the following paragraphs.

2. THE INFLUENCE OF GEOLOGY AND CLIMATE IN THE CONSIDERATION OF METHODS OF SOIL CLASSIFICATION FOR THE TWO REGIONS.

One of the chief objectives of this study is to formulate a system of soil classification and to determine whether the soils of portions of two continents separated by several thousands of miles of ocean and having varied geological and climatic conditions can be classified satisfactorily after the same system. With this principle in mind in the field study of the soils, they have been classified in both regions, as previously recorded, after the New Jersey system, no difficulty in the utilisation of which was encountered in south-eastern England or central New Jersey. Other systems of classification were considered but attempts to apply them were not satisfactory. For example, considering south-eastern England as a whole the climate is everywhere quite equable so that applying the Russian scheme, which is fundamentally based upon climatic zones, one would expect to encounter a zone of soils having uniform profile characteristics. Such, however, is not visible in the field ; on the contrary the type of profile developed depends upon the nature of the parent material, which is a geologic factor. To take an example, the profile of the soils classified in the Hothfield series (derived residually from the Folkestone sands) are without doubt true podsols but when the soils of the Rattle series (which owe their orgin to the Clay-with-Flints) are considered, podsolisation has only taken place to a limited extent, if at all, and it is quite possible that the soils of this series may belong to the Brown earths of Ramann, rather than to the podsol group. Due to the lack of available laboratory data the author is unable to state whether such is a fact, because it was not possible accurately to determine this from field observations. However, even should laboratory analyses indicate that the Rattle Soils are podsolised, the degree of podsolisation differs quite radically from that visible in the Hothfield soils and this differentiation obviously might be explained by the nature of the geologic parent materials concerned. With this in mind, it is quite difficult to consider the parent material a " passive " factor and climate an " active " one in soil formation in south-eastern

England. This degree of podsolisation is also observed in central New Jersey, where the results of the process are easily visible in all the soil profiles, but here again the degree of podsolisation is dependent upon the nature of their parent geologic materials and the author would consider these materials active rather than passive factors in the formation of the soils of the region.

It is therefore concluded that any system of classification applicable to both regions must be one in which geologic factors are dominant and it was for this reason that in classifying the soils the New Jersey system, as previously outlined, was utilised. From the results obtained, it is apparent that this scheme is entirely satisfactory for the purpose in these two regions and it is suggested that the same system might find satisfactory application elsewhere in the British Empire and western Europe.

3. THE INFLUENCE OF GEOLOGY AND CLIMATE ON THE CLASSIFICATION AND DISTRIBUTION OF THE SOILS.

As pointed out previously, in the classification of the soils of any region it is necessary to analyse and study the relative effect of various dynamic factors on the formation of the soils. In both central New Jersey and south-eastern England, since geology has been the dominant factor in this process the outstanding physical and chemical soil characteristics can be traced directly to the geological formations. The various soils in both regions therefore, usually occur in banded areas, extending relative in length and width to the geological formations and in the same general direction. The effect of rainfall and temperature has not been sufficient to alter this close relationship, so that the importance of the nature of the parent materials is further emphasised.

4. THE INFLUENCE OF GEOLOGY AND CLIMATE ON THE SOIL SERIES.

The method by which the soils have been classified into soil series has been outlined previously. It now becomes necessary to study the relation of the geology and climate to the soil series as classified in the two countries. In comparing this relationship, as shown in Table VIII, it is immediately apparent that nearly twice as many soil series occur within a given area in south-east England as within an area of similar size in central New Jersey. This is due to the relative distribution in the two districts of geological formations which are uncovered by superficial deposits, many more such formations occurring in England than in New Jersey. This geologic factor of re-worked materials is extensive on the Chalk and apparent in the re-working of the Sandgate, Hythe, Folkestone and Thanet beds in England while the widespread Pleistocene deposits in New Jersey have extensively covered large areas of underlying Tertiary and Cretaceous deposits forming soils having no relation whatever to the deeper geological materials.

TABLE VIII.

Distribution of Soil Series in Relation to Geological Formation.

District.	No. of Geological Formations.	No. of Soil Series.	Average No. of Soil Series per Geological Formation.
South-east England ..	19	37	1.9
Central New Jersey ..	23	20	.86

With further reference to re-worked materials, Post-Tertiary stream terraces are more extensive in England than in New Jersey, as shown in Table IX.

Climatic factors have likewise affected the soil series, but to a much less extent than the nature of the parent materials. This is indicated clearly in the amount of colluvial material found in English soils and a complete lack of the same materials in New Jersey. This is believed due to differential erosion influenced of course both by climatic and geologic factors, but in these districts principally by rainfall. In New Jersey, erosion is sufficiently severe and of such duration as to carry the eroded materials directly to streams to be subsequently deposited, but in England this process is often arrested before the streams are reached resulting in the formation of soil series which owe their origin to colluvial materials of recent geological age. Table IX shows the relative distribution of colluvial and stream terrace soils in the two districts.

TABLE IX.

Distribution of Soil Series in Relation to Colluvial and Stream Terrace Materials.

District	Colluvial Materials.	Post-Tertiary Stream Terraces.
South-east England	2	8
Central New Jersey	0	2

5. THE INFLUENCE OF GEOLOGY AND CLIMATE ON THE SOIL CLASS (TEXTURE).

The distribution of the soil classes in the two districts and their relationship to the geological formations is shown in Table X. Table XI shows the relative distribution of the soil classes in both districts. A study of these data indicates that sandy soils predominate in central New Jersey, while the heavy soils are more common in south-eastern England. The explanation for this lies in the prevalence of sandy unconsolidated geological formations in New Jersey while in south-eastern England heavy silty and clayey unconsolidated deposits and bed rocks containing, when decomposed, relatively high percentages of silts and low percentages of sands predominate. It is not believed that the climate in either district has radically influenced the soil classes other than perhaps slightly to alter the texture of the surface (A) horizons, by the process of eluviation, and to add heavier materials to the lower (B) horizons by illuviation. This process which forms podsol or podsolised types of soils is generally much further advanced in New Jersey than in south-eastern England. The greater total of rainfall in New

TABLE X.

Distribution of Soil Classes in Relation to Geological Formations.

District.	No. of Geological Formations.	No. of Soil Classes.	Average No. of Soil Classes per Geological Formation.
South-east England ..	19	10	55
Central New Jersey ..	23	12	.52

Jersey no doubt accounts for a portion of this difference. On the other hand, the ground rarely freezes to any great depth in south-east England, while in central New Jersey it usually remains frozen to a considerable depth for long periods during the winter months. The podsolic process is therefore active in England over a longer period in the average year. In discussing this alteration of the texture of the horizons as affected by climatic factors, the nature of the original geologic materials must also receive consideration and as heavy, relatively impervious, materials tending to retard podsolic processes predominate in south-eastern England and more open, lighter textured, pervious materials are more common in New Jersey, the advancement of this process, if entirely due to the nature of the geologic materials, would of course be further advanced in New Jersey. This is actually the fact but on the other hand it is quite certain that the degree of podsolisation in both districts is not entirely due to the influence of either climatic or geologic factors alone, but rather to a combination of the two. In this process again the author is inclined to believe that the geologic factor is dominant.

TABLE XI.

Relative Distribution of Soil Classes.

	No. of Soil Classes.														
District.	Sands.	Fine Sands.	Loamy Sands.	Loamy Fine Sands.	Loamy Coarse Sands.	Sandy Loams.	Fine Sandy Loams.	Gravelly Sandy Loams.	Loams	Gravelly Loams.	Shale Loams.	Stony Loams.	Silt Loams.	Silty Clay Loams.	Clay Loams.	Total.
South-east England	—	1	—	2	1	6	4	—	22	1	—	—	12	6	2	57
Central New Jersey	4	4	2	—	1	9	3	1	9	1	2	2	10	—	—	48

TABLE XII.

Relative Textures of Surface Horizons.

District.	No. of Soil Types Lighter than Loams.	No of Soil Types—Loams.	No. of Soil Types Heavier than Loams.	Total Soil Types.
South-east England	14	22	21	57
Central New Jersey	24	9	15	48

6. THE INFLUENCE OF GEOLOGY AND CLIMATE ON THE SOIL PROFILES.

Before discussing the relation of geology and climate to the soil profiles of the districts it is desirable to have a clear understanding of the modern definition of a soil profile and soil morphology. It must further be remembered that the soil must be considered as a natural body having a definite morphology developed by the force of weathering upon organic and inorganic materials which occupy the surface layers of the earth's crust, and finding expression in the soil profile.

A soil profile is defined as a vertical section of the soil from the surface to the underlying unweathered material. In the description of a soil profile all features characteristic of that soil section are recorded—the texture, colour, structure, consistency, thickness, chemical reaction and any other determinable facts. Profiles are further divided into horizons which are the various layers of the soil section. In a mature soil there are at least three layers designated from the surface downward as the A, B, C horizons. The A horizon is the upper horizon of the soil mass, from which material has been removed by percolating waters. It is generally subdivided into two or more sub-horizons of which the uppermost, or A_0 is not a part of the mineral soil, but the accumulation of organic debris, upon the surface. Other sub-horizons are designated as A_1, A_2, etc. The B horizon is the horizon of deposition to which materials have been added by percolating waters. It is the horizon of illuviation. This horizon also may be divided into several sub-horizons depending upon colour, structure, consistency and other characters. These sub-horizons are designated on B_1, B_2, B_3, etc. The C horizon underlies the B and is the zone of relatively unweathered material. In the upper portions usually some weathered modifications are evident so that sub-horizons are also found in this zone and designated as C_1, C_2, etc. In most cases the C horizon represents the " parent material " being similar mineralogically to that from which the soil was formed. In some cases it may be a stratum, or geological formation of different material.

It should also be pointed out that it is only in mature soils that profile characteristics are developed. Soils of recent formation or immature soils (Endodynamomorphic soils) show few or no profile characteristics.

In studying the relation of geology and climate to the profiles of the two districts it is important to note that a regional profile is developed in all mature soils in both districts. These regional profiles are characterised by A horizons relatively lighter in texture than the B horizons. The texture of the C horizons is dependent upon the nature of the parent geological materials. In central New Jersey, as shown in Table XII the A horizons are generally more sandy in texture than those found in south-east England. The textural differences between the A and B horizons are much better marked in New Jersey as has previously been pointed out. The process of podsolisation is also much further advanced in New Jersey. In considering the whole profile, sandy layers predominate in New Jersey while heavy horizons are numerous in south-east England. It is believed that this difference is chiefly due to the nature of the geological materials from which the soils of the respective districts have been formed.

TABLE XIII.

Chemical Reaction of Soil Profiles.

District.	UPPER HORIZONS (A).		LOWER HORIZONS (B).		
	Acid.	Neutral or Alkaline.	Acid.	Neutral or Alkaline.	Variable.
South-east England ..	23	14	14	20	3
Central New Jersey	18	0	18	0	0

7. THE INFLUENCE OF GEOLOGY AND CLIMATE ON SOIL REACTION.

The chemical reaction, that is the acidity, alkalinity or neutrality of the upper and lower horizons of the soils of both districts is shown in Table XIII. In New Jersey all the surface and lower horizons are acid in reaction while in south-eastern England many soils are alkaline or neutral in the upper or lower horizons or both. This is a most striking relationship and points out very clearly the close relation between the reaction of the geologic materials and the soils. All the geologic materials in New Jersey are acid in reaction, and so are the soils, while in south-east England geological formations basic in reaction give rise to neutral or alkaline soils and formations of acid reaction develop soils having an acid reaction. Climatic factors have also influenced the soil reaction, especially in south-eastern England where the forces of temperature and rainfall have not been of sufficient intensity extensively to leach alkaline materials from the upper horizons of soils formed from basic geologic materials. As previously related, there are no geologic materials in central New Jersey having an alkaline reaction but in adjoining areas of New Jersey having climatic conditions identical with the area under discussion, the author has examined and classified sedentary soils derived from limestone which, in their upper horizons, have a neutral or acid reaction and further, the texture and profile character of these soils resemble very closely many soils of alkaline reaction in south-eastern England. It is therefore concluded that the reaction in soils derived from materials of similar basic materials has been much more altered by climatic factors in New Jersey than in south-eastern England.

XVI. SUMMARY.

1. An introduction and review of the literature on the subject is presented.
2. The various methods of soil classification are discussed. The New Jersey system is discussed at length.
3. Recent contributions in soil classification in the British Isles are briefly reviewed.
4. The general geographic location, climate, geology and physiography of the areas under study are described and the scope of the subject stated.
5. The methods of study used are presented.
6. The geographic location, physiography, geological formations, natural drainage and climate of the central New Jersey area are described in detail.
7. The soils of the central New Jersey area are classified into series, classes and types according to the New Jersey system and each important soil series is described in detail.
8. The geographic location, physiography, geological formations, drainage and climate of south-eastern England are described in detail.
9. The soils of south-eastern England are classified into series, classes and types after the New Jersey system and each important soil series is described in detail.
10. The dynamics of soil formation is discussed.
11. The influence of geology and climate in the consideration of methods of soil classification for the two districts is presented.
12. The influence of geology and climate on the classification and distribution of the soils of south-eastern England and central New Jersey is shown.

13. The influence of geology and climate on the soil series of both districts is discussed.
14. A discussion is offered upon the influence of geology and climate on the soil class (texture) of the soils of the two regions.
15. Soil profile and soil morphology are briefly discussed and the influence of geological and climatic factors on the profiles of the soils of both districts presented.
16. The influence of geology and climate on the reaction of the soils is discussed.

XVII. CONCLUSIONS.

1. The soils of central New Jersey occur in a belted arrangement closely related to the geological formations.
2. The soils of south-eastern England also occur in belted zones corresponding to the geological formations.
3. Everywhere throughout the world certain dynamic forces are constantly at work in soil formation.
4. In the same soil region certain of these forces are dominant and others recessive.
5. Geology has been the dominant factor in soil formation and distribution in south-eastern England and central New Jersey.
6. Geological factors therefore receive first consideration in classifying the soils of these districts.
7. The soils of south-eastern England and central New Jersey can both be satisfactorily classified by the New Jersey system.
8. There is a direct relationship between the geological formations and climate both in south-eastern England and in central New Jersey and the distribution of the soil series.
9. Nearly twice as many soil series occur in south-eastern England as in central New Jersey.
10. Re-worked geological materials have played an important part in the distribution of the soil series in both districts. This is more important in New Jersey.
11. Soils of colluvial origin occur in south-eastern England but are not found in central New Jersey.
12. Soils formed from Post-Tertiary stream terraces are more prominent, and much more numerous and extensive in south-east England.
13. There is a direct relationship between the geology and climate and distribution of soil classes (texture) in the two districts.
14. More soil classes occur in central New Jersey than in south-eastern England.
15. Podsolisation in general is further advanced in the soils of central New Jersey than in south-eastern England.
16. A similar regional profile is developed in both districts. This consists typically of A horizons relatively lighter in texture than the underlying B horizons and overlying C horizons heavy or light in texture depending upon the nature of the geologic (parent) material. The textural relation between the A and B horizons is much more marked in central New Jersey than in south-eastern England.

17. Sandy profiles predominate in New Jersey.
18. Heavy profiles, containing high percentages of silts and clays, are more numerous in south-east England.
19. There is a direct relation between the texture of the soil profile and the geological formations in each district.
20. Climate and geology have a direct relationship to the soil reaction in both districts. No alkaline soils occur in central New Jersey while they are quite common in south-eastern England. Climate factors have altered soil reaction more in central New Jersey than in south-eastern England.

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STUDIES IN SOIL CULTIVATION.

VI. THE PHYSICAL EFFECT OF SHEEP FOLDING ON THE SOIL.

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(With Five Text-figures.)

INTRODUCTION.

THE beneficial effects on light land produced by folding with sheep have been known for a long time: the value of this system of husbandry was crystallised in the phrase "the golden hoof of the sheep." The results were put down partly to organic manure which was evenly distributed over the folded area, and partly, or perhaps more definitely, to the mechanical consolidation of the soil produced by the pressure of the sheep hooves. Whatever the explanation, the system enabled light land to be kept under arable cultivation and in particular to produce malting barley of the highest quality. The full success of the system, however, demanded that the sheep should be directly profitable to the farmer, and in recent years this source of income has been uncertain. In theory it should be possible to simulate closely the effect of sheep folding by ploughing in a green crop and by consolidating the soil with suitable implements. Instances are known when this treatment has failed, and one was recently brought to our notice by Sir Daniel Hall in a private communication.

It was evidently desirable to make a study of the physical effect of sheep treading on the soil in order to obtain some idea of the kind and extent of the consolidation produced and also to see how far any such compression survives the subsequent cultivation operations. The present paper is an account of the results obtained.

EXPERIMENTAL METHOD.

It appeared desirable in the first instance to measure the variation of consolidation with depth on the trodden land and to compare it with a portion of the same area from which the sheep had been excluded. A possible method was to take the weight of known volumes of successive

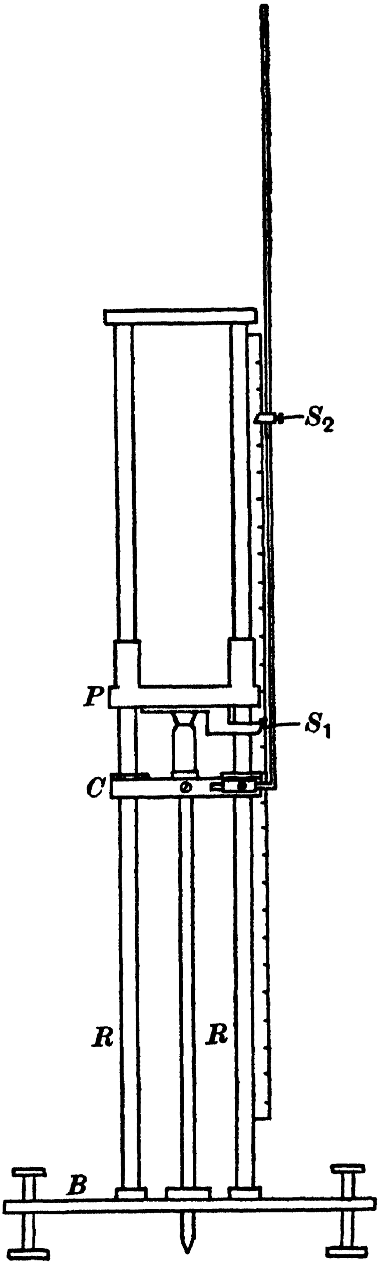


Fig. 1.

depths at intervals during the treading. There were obvious disadvantages in this method. The volume can only be approximately measured, and considerable disturbances are inevitable while the sample is being taken. A number of measurements would also have to be made for each depth in view of possible soil heterogeneity (1). It appeared preferable to confine the observations to a small area and to disturb the soil as little as possible in the process. The method adopted was to measure the resistance offered by the soil to the passage of a vertical rod. An apparatus on the principle of a pile driver was made to our design by Messrs Gallenkamp for the purpose and is shown diagrammatically in Fig. 1.

The frame of the instrument consists of a base plate *B* fitted with levelling screws and three vertical steel rods *R*, two of which serve as guides for the weight platform *P* and the cross-piece *C*. A centimetre scale is mounted on one guide. The steel plunger driven into the soil is rigidly attached to *C*, which is fitted with two ball races. The latter, together with a third ball race on the base plate, prevent any lateral movement of the rod. The platform *P* fits the guides very loosely so as to prevent contact with them as it falls, and the guides were kept oiled to diminish any frictional effect should contact occur. The height through which the platform falls is adjustable, and is kept constant in any experiment by means of the two stops S_1 and S_2, attached to the platform and plunger respectively.

In commencing an experiment the point of the plunger is supported by a piece of thin sheet metal resting on the soil surface, and the apparatus is accurately levelled. The initial reading is taken, the platform raised and the sheet metal withdrawn as the platform is allowed to drop. Readings are then taken for successive falls of the platform until the required depth has been reached.

EXPERIMENTAL RESULTS.

The work was carried out at the Woburn Experimental Station on light sandy soil of a nature customarily folded with sheep.

Preliminary trials of the apparatus were made on various areas which appeared uniform to visual inspection. Considerable variation was found from one experiment to the next even though the points were only a few inches apart. These measurements demonstrate the existence of rapid variation of soil resistance of the same nature as the larger scale measurements of soil heterogeneity made with a dynamometer (1), but it must be remembered that the very small volume of soil concerned in each experiment gives full opportunity for any fortuitous irregularities to show in the particular curve. Extra consolidation, due to a horse's hoof or the passage of a cart wheel at an earlier date, or extra looseness in the tilth owing to a small depression incompletely filled in, are typical examples of such irregularities.

For comparative purposes it was necessary to take the average of a number of measurements within a small area, and there was no justification for excluding apparently discrepant curves. The following results, therefore, refer to the mean of nine curves taken systematically within an area of approximately 6 ft. square. Two plots 6 ft. square and 12 ft. apart were selected within the area on which sheep were to be folded. Penetration measurements (set A) were made on October 30, 1929, and one plot was then fenced off as a control. The fold was approximately one-fifth of an acre and carried 44 lambs from November 14th to November 18th, and 88 ewes for 4 days from November 21st to November 25th.

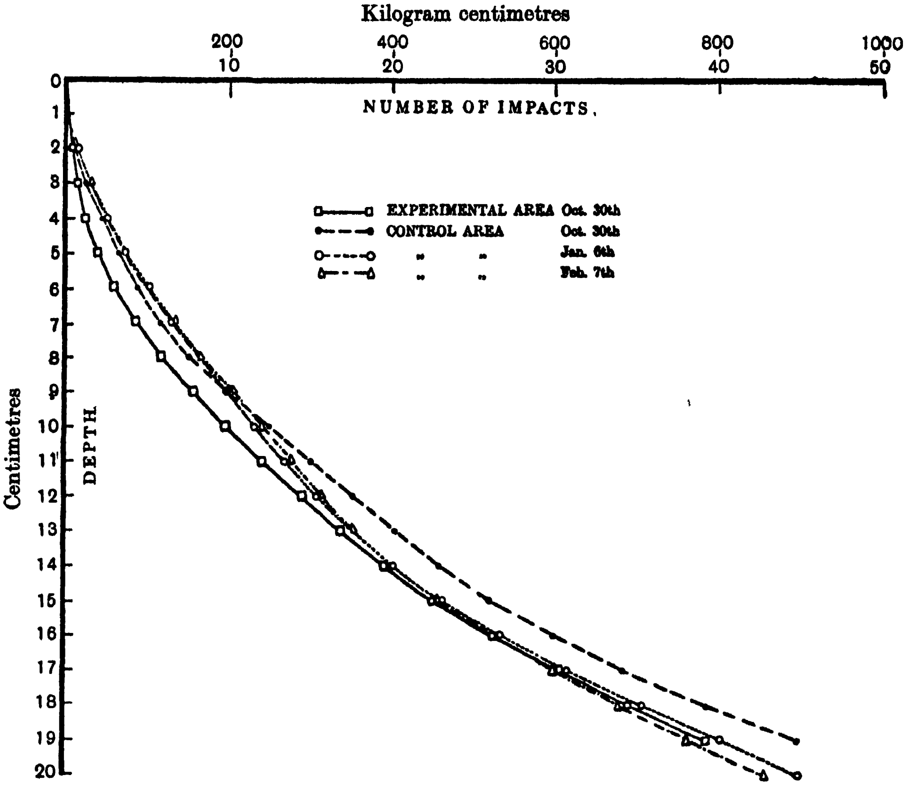


Fig. 2.

The second set (B) of penetration measurements were made on

January 6th, and the area was left undisturbed until February 7th when a third set (C) was taken immediately before the land was ploughed. To ascertain the effect of ploughing on the trodden and control plots sieving tests were carried out before and after the operation in the manner described in the fifth paper of this series (2). The sieving tests were repeated on March 14th, the area having been undisturbed since February 7th.

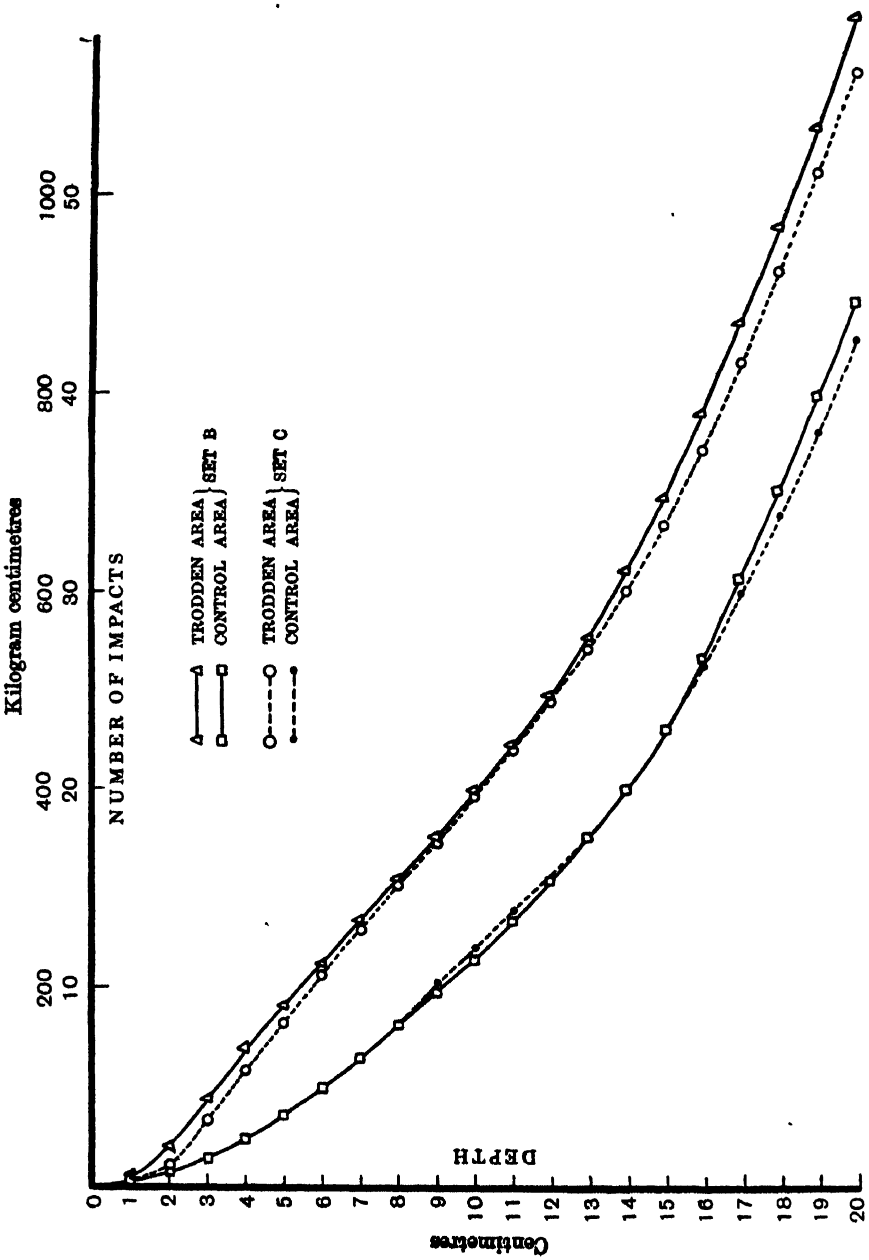
The reliability of the measurements is shown by Fig. 2, giving the average curves for set A (both plots) and for the control area in sets B and C. The four curves lie closely together, although one of them refers to a plot 12 ft. away from the other and an interval of over 3 months separated the first and third sets. Fig. 2 also shows that equal energy increments produce progressively decreasing increments of descent of the rod.

The effect of sheep treading is shown by comparing the two curves for set B (Fig. 3), the region affected being roughly between the 2 and 10 cm. levels, where the trodden-area curve is concave downwards. Below 12 cm. the curves are sensibly parallel, showing that sheep treading has no appreciable consolidating effect below this depth. That the consolidation persists for at least a month after removal of the sheep is demonstrated by the agreement of the curves of set C with those of set B.

The treading effect can be seen in more detail if the derived curves are considered. These are shown in Fig. 4, in which the energy needed to give 1 cm. penetration at different depths is plotted against those depths. It was considered sufficient for the present purpose to read off from the mean curves the energy required to increase the penetration from n to $(n+1)$ cm., and to plot this value against $(n+\frac{1}{2})$ cm. As the derived curves for the control and trodden area in set B are similar to the corresponding curves in set C, a single curve has been drawn for each plot through the two sets of data.

Considering the control plot first the energy required for unit penetration increases with depth. For the first 12 cm. the curve is concave to the depth axis, and would suggest that a limiting value of the energy required to produce 1 cm. penetration is reached at a depth of 10–12 cm. At 12 cm., however, the energy required begins to increase and does so regularly for the remainder of the depth range investigated. From this it would appear that the 12 cm. depth is connected in some way with cultivation operations, *e.g.* depth of ploughing.

For the trodden plot, on the other hand, the energy required rises


Kilogram centimetres
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400
600
800
1000
NUMBER OF IMPACTS
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20
30
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50
TRODDEN AREA } SET B
CONTROL AREA } SET B
TRODDEN AREA } SET C
CONTROL AREA } SET C
DEPTH
Centimetres
0
1
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Fig. 3.

rapidly to a maximum at a depth of 3½ cm., after which it falls and passes through a minimum at 8 cm. depth. From 12 cm. onwards the curve is parallel to that for the control area from the same depth, although the values are higher. The latter fact is not due to a direct compression effect at this depth: there will be frictional forces exerted by the soil on the length of rod with which it is in contact, and these will be greater the more tightly the soil is packed around the rod. Similarly a compression on the trodden area must mean that a depth of 15 cm. say on this area is relatively deeper into the subsoil than a depth of 15 cm. on the untrodden. Both these effects would tend to give a higher energy requirement for 1 cm. penetration on the trodden than on the control area. In any case a comparison of the two curves shows that the main and probably the total effect is confined to the first 10 cm.

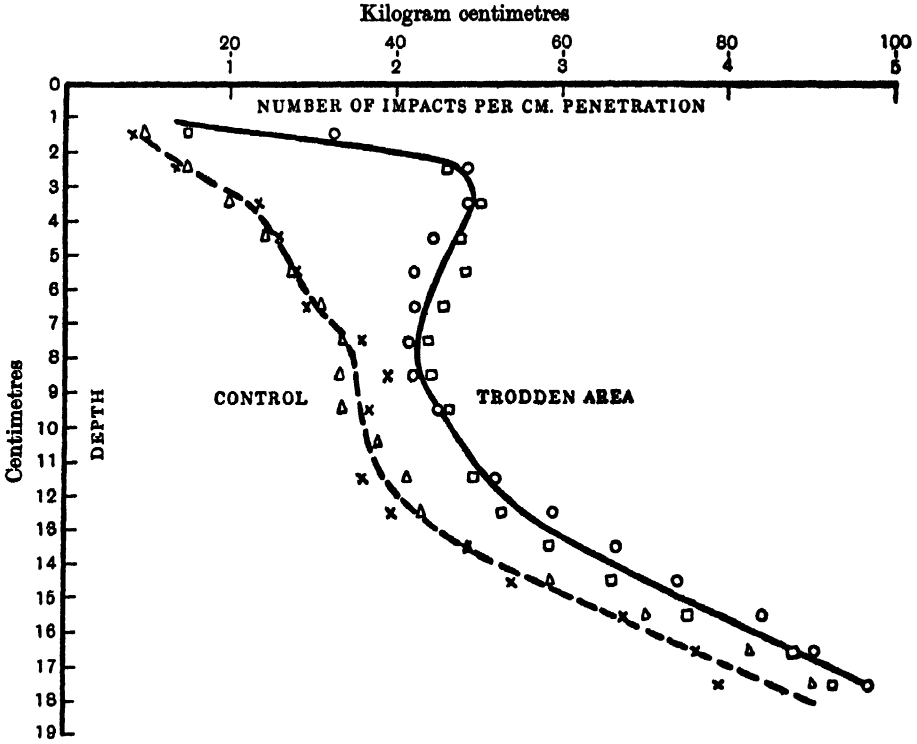

Fig. 4.

The results of the sieving tests are shown in Fig. 5. Four sieves were used, three of which had square apertures of 1·5 in., 0·5 in. and 0·25 in., while the fourth was a 3 mm. round hole type. These divided the soil into five samples A to E. Fraction A consists of lumps retained by the

1·5 in. sieve, B those retained by the 0·5 in. sieve, etc., and E the soil crumbs passing the 3 mm. sieve. The ordinates represent the amounts of these fractions expressed as a percentage of the total weight of the sample. Before ploughing the trodden area gave a much higher per-

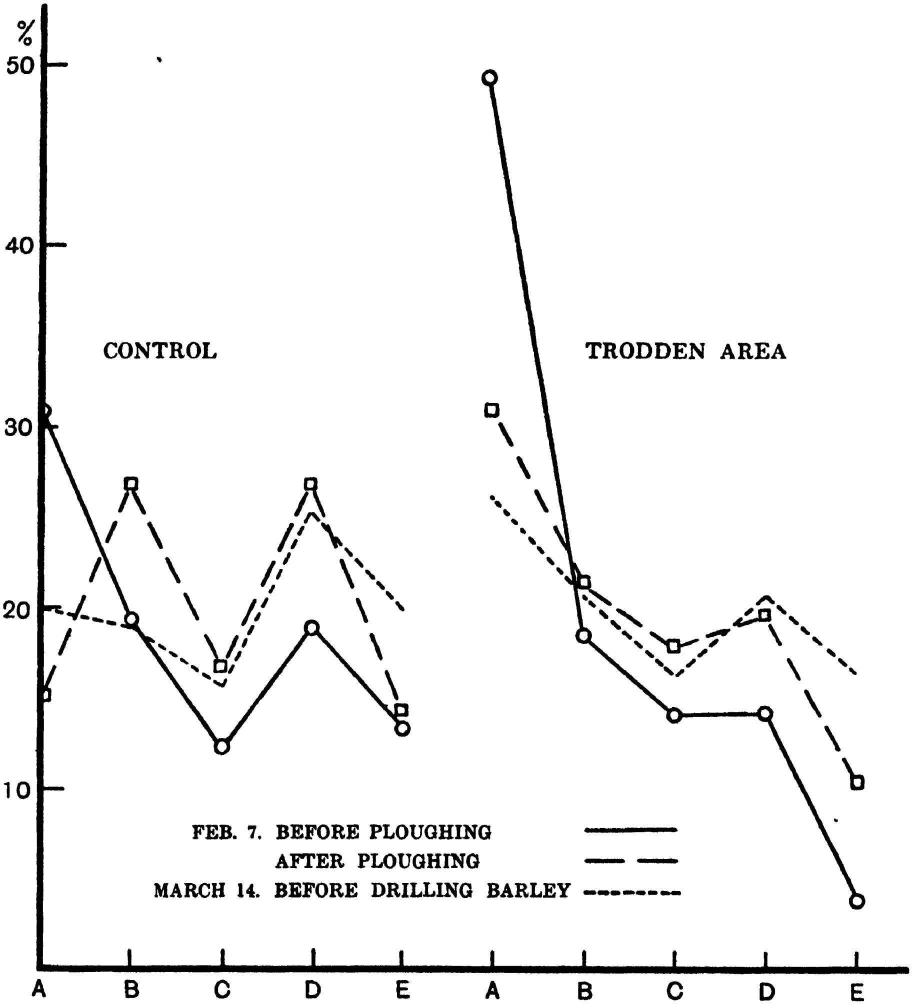

Fig. 5.

centage of the first fraction and lower percentages for the two finest fractions when compared with the control. Ploughing increased the percentage of the four finer fractions for both plots, but the trodden area had still a smaller percentage of the two finest fractions. The difference persisted as is shown by the readings for March 14th when the barley was sown. Thus the ploughing broke up the soil along lines of weakness, but owing to compression these were fewer on the untrodden than on the trodden area and the physical effect of folding was not completely destroyed.

The type of compression obtained by sheep folding is very similar to that produced by rollers, as far as can be judged from the results of compression tests. Mangelsdorff (3) cultivated soil to a depth of 25 cm. by means of a rototiller, and investigated the compression caused by various types of rollers. For the rototilled soil the forces required increased with depth: after rolling, however, the curves exhibited the maximum and minimum which have been obtained in the derived curves in the present experiment. These occur at greater depths than those observed after sheep folding, but this is due to the very loose condition in which a rototiller leaves the soil.

It has been suggested that folding would increase the moisture-holding capacity of the lighter soils, but moisture determinations on two occasions in the present experiment show that the two plots did not differ greatly. Actually the samples from the untrodden plot were slightly the wetter. The coarser structure of the soil after folding may decrease the loss of nutrient by leaching, which is an important factor on light soil, but further work is necessary before any definite statement could be made on this problem, and whether this loss would be less than from a green manure ploughed in, is again a very debatable point.

SUMMARY.

1. The consolidation in a light soil produced by sheep folding has been investigated.
2. The soil affected extended to a depth of 10 cm., the maximum compression occurring at a depth of 3–4 cm.
3. The consolidation produced was not totally destroyed by ploughing and was still apparent 5 weeks later when the seed was drilled.

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SOIL PHYSICS IN RELATION TO METEOROLOGY

By Dr. B. A. KEEN, Assistant Director, Rothamsted Experimental Station.

(The G. J. Symons Memorial Lecture delivered on March 16, 1932.)

INTRODUCTION

The physical properties of the soil have received greatly increased attention from research workers in the past ten to fifteen years, and the importance of these studies is now fully recognised. Apart from their intrinsic research interest—and the physical properties of moist, porous colloidal materials, such as soil, have been curiously neglected by physicists—these studies have two other important applications : the first is the development of a scientific basis for the study of the highly developed but empirical art of soil cultivation, and the second is to provide reliable information about the environmental conditions in the soil for the guidance of those engaged in the study of the soil micro-organisms, which play so great a part in maintaining its fertility.

The properties of the soil are very closely related to its moisture, temperature and air supply, and thus provide direct contacts with certain branches of meteorology. The amount and distribution of moisture in the soil are the resultants of rainfall, evaporation and percolation ; the march of soil temperature is directly controlled by insolation and radiation ; changes in the atmosphere affect the composition of the atmosphere in the pores of the soil. Nor is this all ; moisture, temperature and atmosphere are not independent variables ; for example, changes in moisture content have important and unexpected effects on soil temperature. There is, in fact, a most interesting border-land between soil physics and meteorology, and some features of it are discussed in detail below.

SOIL CLASSIFICATION IN RELATION TO CLIMATIC ZONES

The division of the world into broad climatic zones is familiar to the meteorologist and the physical geographer. But only in the last few years has it been realised that the soils of the world also fall into a few broad groups whose distribution is determined in no small degree by the climatic zones. The discovery was first made by the Russians who had the advantage of working in an extensive country showing a wide range of climatic conditions. They found that although the geological origin of the soil had an influence on the type of soil formed, the effect of the climate was more important. Owing to language difficulties the Russian conception of soil groups did not reach western Europe for some time. In fact, it was not until the formation of the International Society of Soil Science, which gave opportunities for soil workers in different countries to discuss common problems, that the Russian conception gained general acceptance, and a satisfactory basis was evolved for mapping the great soil groups of the world. The characterisation is made in the field by examining a vertical section or " profile " of the soil, and recording the appearance and structure of the different layers, which may be more or less marked ; samples are also taken for chemical and physical examination. The main effects observed are those due to rainfall—or rather to percolation—and evaporation, which cause the solution and interaction of soluble substances and their transport within the soil mass, and thus produce definite and recognisable results in the appearance of the profile. Rainfall, and temperature, which will affect the amount of evaporation, are therefore the meteorological factors most closely connected with soil properties. In Russia the rainfall decreases from north to south and the temperature increases in the same direction. Hence, the effects of percolation are predominant in the north and evaporation in the south, giving conditions favouring a wide variation in the composition of the clay fraction of the soil. This was no doubt the clue which enabled the Russian investigators to arrive at their generalisation. In the north, with high rainfall and low temperature, percolation is predominant, and the soil becomes acid because the soluble salts and bases are carried down to lower levels. There is a surface layer of acid humus material, and the sesquioxides and other soil constituents are washed down to the lower layers of the soil. With a higher temperature the grey and brown forest soils are formed. Here the humus layer is thicker and the acidity is much less developed; there is also less evidence of downward transportation of soluble soil material. With still higher temperatures and reduced rainfall the well-known fertile black earth or Chernozem type is developed, characterised by a higher proportion of humus extending deeper into the soil, and having a neutral reaction. Further south with still higher temperature and reduced rainfall the type changes again through chestnut-coloured soils to grey desert or semi-desert types in which the intense evaporation is responsible for keeping salts and decomposition products within the upper layers and may even bring them to the surface where they form crystalline deposits. But rainfall and temperature have not always the inverse relationship just mentioned. In the United States, for example, rainfall and temperature tend to increase together; hence the distinction between conditions in which either percolation or evaporation predominates becomes less sharp, and the composition of the clay fraction becomes much more constant. Thus the Russian system which focusses attention on the rainfall was found not to answer completely when applied to the United States soils. It was necessary to consider both temperature and rainfall. The relationship has recently been worked out in detail.[1] It is known that the complex aluminosilicates forming the bulk of rock material gradually decompose under the action of water and carbon-dioxide, and that the ultimate result in humid regions is for the alkaline silicates to be washed downwards. The molecular ratio of silica to alumina (SiO_2/Al_2O_3) therefore falls. By applying statistical methods of examination, Crowther showed that the values of this ratio for American soils were negatively correlated with rainfall and positively with temperature. An increase of 1°C. in the mean annual temperature raised the ratio by the same amount as an extra 4 cm. of rainfall lowered it. The mathematical separation of the two variables is, in a sense, a device for measuring the effect of percolation water on the ratio, as no data for percolation were available. It is of interest to note, therefore, that the relationship is confirmed by the actual records of the percolation gauges at Rothamsted which have been in use since 1870. Crowther found that a rise of 1°C. in the mean annual temperature lowered the percolation to the same extent as a reduction in the mean annual rainfall of 3·4 cm.; the value is in good agreement with that found from the American data.

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[1] Crowther: *London, Proc. R. Soc.*, B. 1930, **107**, pp. 1-30.

We may regard this brief outline of soil and climate inter-relations as a general introduction to the more detailed consideration of the physical properties of soil, in which we shall find a very wide and complicated range of factors.

SOIL TEMPERATURES

I do not propose to deal in any detail with either the agricultural or purely meteorological aspects of soil temperature as the broad outline is generally known. I intend to take instead the physical aspects of the problem, and to deal with the transmission of heat in a porous or granular moist material. In general, the soil surface is subjected to a daily rise and fall of temperature, and the amplitude of this wave increases from winter to summer. At a depth of about three feet the daily temperature fluctuations are inappreciable, and only the annual seasonal changes of temperature are measurable. Whether we consider the daily or the annual variations, there is a systematic ebb and flow of heat. Thus in the night-time for the daily wave, and the autumn and winter for the annual wave, the upper part of the soil is cooler than the lower and there is an outward flow of heat from the interior. Conversely, in the day-time, and in the spring and summer the flow of heat is towards the colder interior. Rambaut[2] has measured the mean monthly temperatures at Oxford in gravel soil under grass, and his results, shown in Fig. 1, give a clear picture of the temperature wave. The diminution of amplitude with depth is brought out; the range of temperature at 2ft. is from 40°F. to 62·5°F., while at 10ft. it is much less, from 46·5°F. to 56°F. The ebb and flow of heat is also shown; over the period November to March the soil is warmest at the lowest depths and the flow of heat is outwards, while from May to September the reverse is the case. The periodic cyclic nature of the process is reflected in the six-monthly symmetry in the diagram, each monthly curve being the mirror image of the one obtained six months later. If the February curve is imagined to sweep over the diagram by taking successively the positions of the other curves, a good general idea of the cycle of changes is obtained. Fig. 1 shows the change in temperature with depth, for certain given times. It is instructive to plot the data in another way and to show the changes in temperature with time for certain fixed depths. But instead of using Rambaut's data for this purpose it will be more convenient to take values obtained at shallower depths of soil which show the daily temperature waves. Fig. 2 gives the temperature-time curves for soil at Giza, Cairo,[3] the depths ranging

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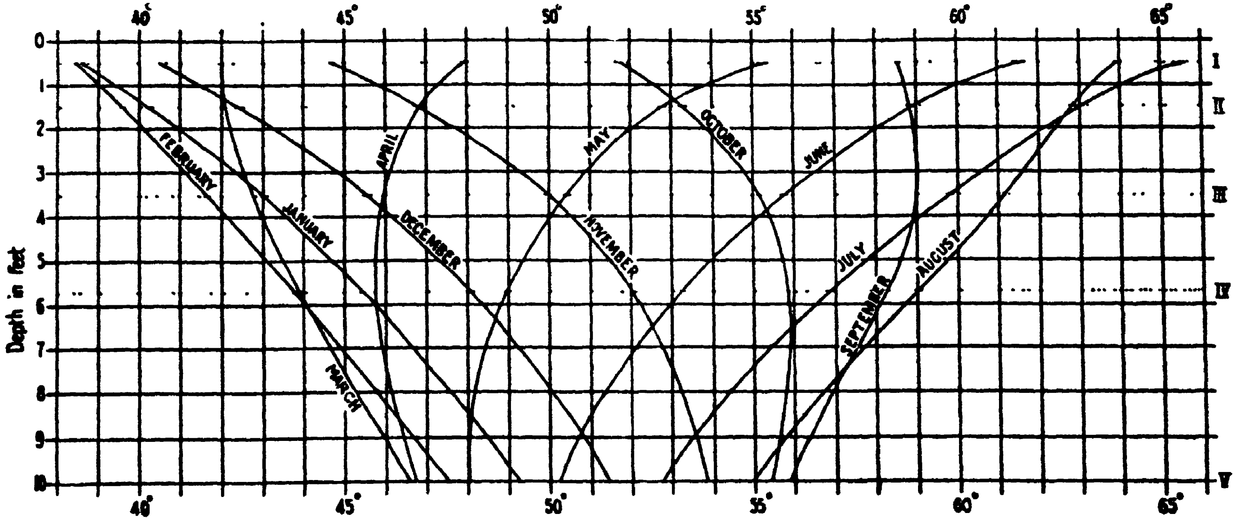
[2] Rambaut: Underground temperature at Oxford as determined by means of five platinum resistance thermometers, Nov. 1898 to Oct. 1910. *Radcliffe Obs. Meteor. Obs.*, **51**, 1911-5.

[3] McKenzie-Taylor and Williams. *Egypt, Min. Agric. Tech. and Sci. Service*, 1924. *Bull.* No. 40.



The positions of the thermometers are indicated by the Roman numerals.

FIG. 1.—Soil temperatures in °F. in grass-covered soil.

(*From "Radcliffe Observations," Vol. 51, by permission of the Oxford University Press.*)

from the surface to 20 cm. We see how rapidly the amplitude of the wave decreases with increase of depth, and the slow rate of penetration is shown by the times at which corresponding points on the curves are reached as the depth increases; thus the maximum temperature at the surface occurs at about 12h., while at the 20 cm. depth it is not reached until 24h. A glance at the curves shows that the oft-used phrase "the temperature of the soil" is devoid of any real meaning unless both the time and depth are recorded. But although the sequence of temperatures at different times and depths looks very complicated the general features are expressible by the theory of heat flow in a conducting material worked out by Fourier in a classical study. For us the real interest lies in the modifications imposed on the theory by the porous nature of the soil and the distribution of water throughout its mass.

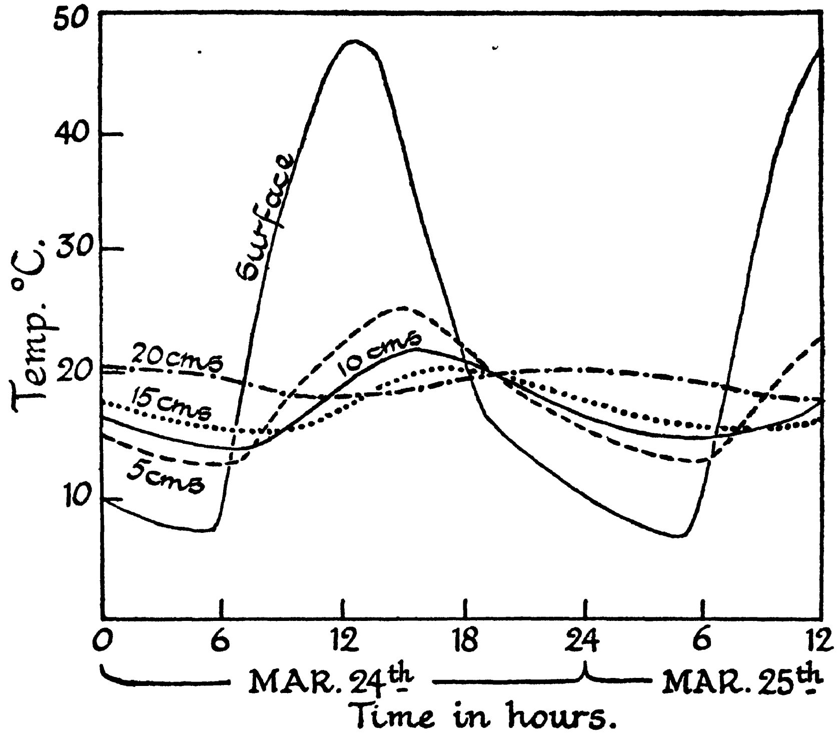


FIG 2.—Soil temperatures at Giza, Cairo.
(*From the "Technical and Science Service Bulletin of the Ministry of Agriculture, Egypt," No. 40, 1924.*)

The fundamental differential equation for heat conduction in the circumstance we are now considering is

$$K\frac{\delta^2\theta}{\delta x^2}=c\frac{\delta\theta}{\delta t} \text{ or } k\frac{\delta^2\theta}{\delta x^2}=\frac{\delta\theta}{\delta t} \quad . \quad . \quad . \quad (1)$$

where $k=K/c$. In this equation θ, x, and t are respectively temperature, depth and time, K is the heat conductivity, and c is the volume specific heat, *i.e.*, the product of the specific heat of the soil material itself and its apparent density. The quantity k, is the diffusivity and is the temperature change produced in unit volume of the soil by the amount of heat flowing in unit time through a unit cube having unit temperature difference between two opposite faces. As the diffusivity determines the temperature change in a soil layer when heat is conducted from an adjacent layer, it is convenient to use it rather than K and c when dealing with soil temperature changes.

Now if the soil surface were subjected to a continuous series of identical temperature oscillations, after a time a state of dynamic temperature equilibrium would be reached in the body of the soil, and the temperature at any point would oscillate between certain fixed values. If a simple harmonic temperature oscillation be chosen then the solution of the differential equation is

$$\theta=\theta_0 e^{-2\pi x/\lambda} \sin 2\pi\,(t/T-x/\lambda) \quad . \quad . \quad . \quad (2)$$

This equation defines the temperature θ at any depth x, and time t, in terms of functions of T and λ. T is the time period of the wave, *i.e.*, one day in the case of a diurnal wave, and λ is the "wave length," *i.e.*, the distance between points at which maxima (or minima) of temperatures occur simultaneously. The diffusivity is related to λ and T by the equation $k=\lambda^2/2\pi T$.

Equation (2) contains a sine and an exponential term. The former shows that at any given depth x, the fluctuation of temperature is an image of that at the surface, *i.e.*, is also simple harmonic, while the exponential term shows that the amplitude of this fluctuation falls off with depth.

When actual records of soil temperature are examined from the above standpoint it is seen that they do not obey the simple theory. The curves in Fig. 2, for example, are not simple harmonic in shape, and their form changes with increasing depth. Fourier's full theory applies however to the propagation of any periodic temperature wave, no matter how complex its form may be, since the function can be resolved into its component simple harmonics, each of which is propagated in accordance with equation (2).

But although it would be possible to examine curves, such as those of Fig. 2, by this means it would scarcely be worth while, for the soil material in which the temperature wave is propagated is not necessarily uniform in composition or in arrangement of the particles, and in addition it contains moisture. Hence, the values K and c in the fundamental equation of heat flow are not constants, but may themselves be functions of the temperature. Indeed from both the physical and agricultural viewpoint these aspects are more interesting than the formal problem of heat propagation in a uniform material.

Moisture in the soil will have two obvious effects on the temperature. The first is in increasing the specific heat per unit volume (the quantity c), for a dry soil has a specific heat of about one-fifth that of water; hence the well-known fact that a given amount of heat raises the temperature of wet soil to a lesser degree than dry soil.

The second effect is a consequence of the fact that in soil the rock material is not continuous, but in particle form. The conductivity in the continuous rock form is about seven times higher than that of water, but in particle form, owing to the poor thermal contact between the particles, it is only about one-third to one-half that of water. Hence, additions of water to a dry soil should increase the conductivity. Patten's[4] experiments showed that this was so; but he obtained the surprising result that the conductivity of moist soil was sometimes increased not only up to the value of water, but beyond it. The explanation of this paradox is found by considering the manner in which water distributes itself in soil. The effect of surface tension is to cause the water to form annular rings around the points of contact between the soil particles, which are thus drawn into firmer contact and therefore the higher heat conductivity of the soil material becomes more effective. In addition heat can now pass from particle to particle by conduction through the thin moisture ring instead of by convection and radiation across the empty pore-space. At higher moisture contents the low heat conductivity of water becomes predominant, and the value falls.

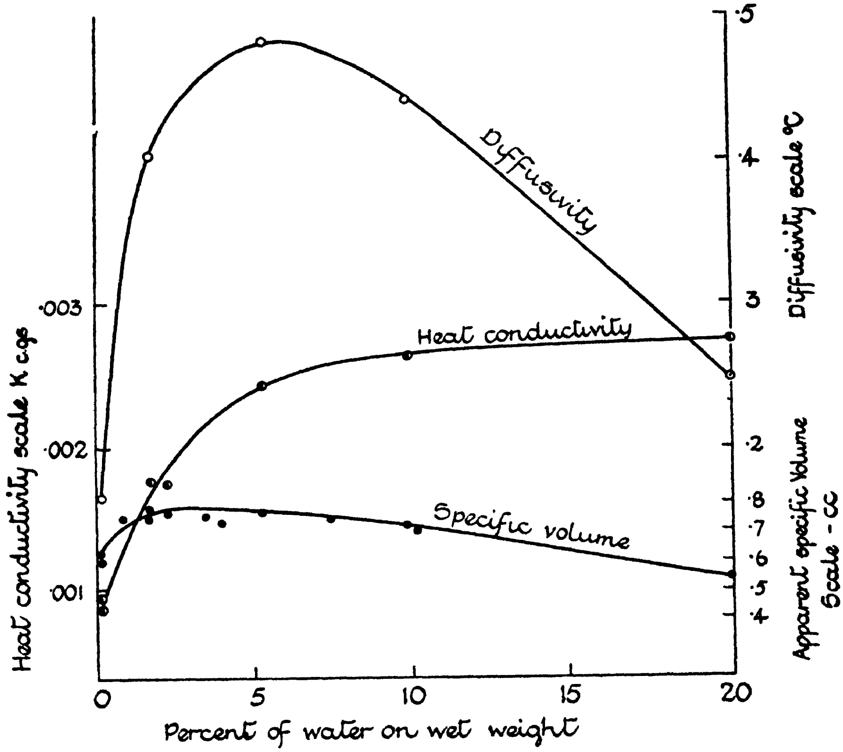


FIG. 3.—Effect of moisture content on volume and thermal behaviour of a coarse quartz powder.

(*From U.S. Dept. Agric., Bureau of Soils Bulletin No.* 59, 1909.)

Patten's experimental work could not be carried out by applying heat until the steady stage was reached, for long-continued heating would have caused movements of moisture and thus have vitiated the results. The measurements were made during the initial unsteady stage of the heat flow, by noting at frequent intervals the readings of a number of thermometers disposed at different distances from the source of heat. From these readings the values of $\delta^2\theta/\delta x^2$ and $\delta\theta/\delta t$ were obtained, and separate determinations of c were made, from which K could be determined by using equation (1). Typical results are shown in Figs. 3 and 4. It will be observed that quartz powder gives more regular results than soil, possibly because of the greater uniformity of size of the particles. The interesting feature of the results is the marked maximum in the diffusivity, showing that there is an optimum moisture content for the rate of rise of soil temperature.

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[4] Patten. *Washington, D.C., Bull. U.S. Dept. Agric. Bur. Soils*, No. 59, 1909.

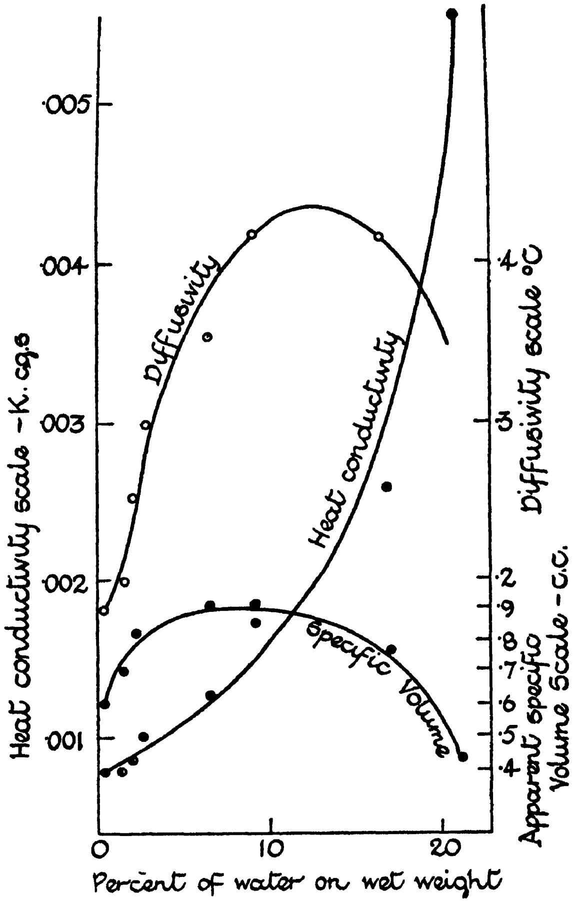

FIG. 4.—Effect of moisture on volume and thermal behaviour of a fine sandy loam. (*From U.S. Dept. Agric., Bureau of Soils Bulletin No. 59, 1909.*)

The effect of moisture content on diffusivity is therefore of considerable interest. It was examined in detail by Callendar and McLeod[5] in Canada, for the soil layer between the 20in. and 100in.

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[5] Callendar and McLeod. *Ottawa, Proc. R. Soc., Canada*, 2nd Ser., 3, 1897-8, pp. 31-49.

depths. They employed a graphical method similar to that of Patten and obtained the average diffusivity for selected periods of the year. The lowest value was 0·00156 during February, and as the soil was frozen it is probable that this represents the value for pure thermal conduction uncomplicated by actual movements of water. Any values higher than this are therefore not true diffusivities in the sense of equation (1), and the extent of the increase is a measure of the heat transfer by percolation of water. The manner in which the diffusivity varied over the year is shown in Fig. 5. There is evidently a seasonal variation, the value being greatest in spring and autumn when soil temperature is changing more rapidly and bulk movement of soil water is most likely to happen. In the summer lower values are obtained because of reduced percolation, and the drying out of the top soil to a mulch

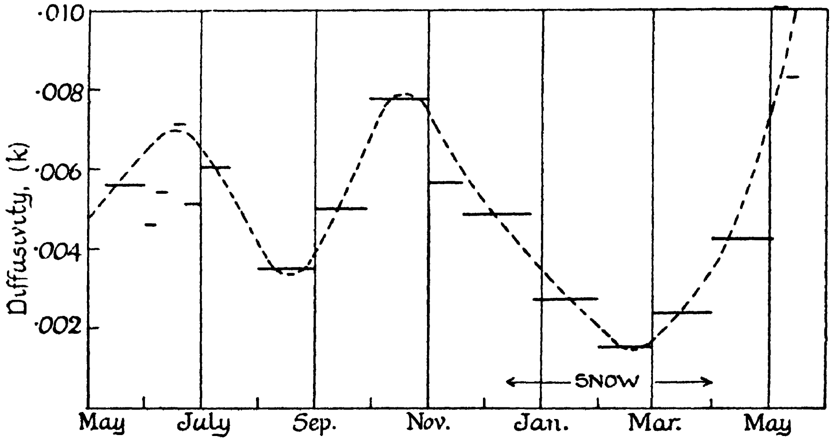

Fig 5.—Diffusivity for soil at Montreal between 20in. and 100in. depth. Horizontal lines represent average value of *k* for period covered. Dotted curve shows probable average value of *k*.
(*From the " Transactions of the Royal Society of Canada."*)

of low diffusivity. Over the year the average value is 0·0036. Similar calculations can be made for temperature readings obtained by McKenzie Taylor and Williams in sand near Cairo, and for the upper 8in. of soil at Rothamsted. The diffusivity values are respectively 0·0032 and 0·0041. Hence in moist temperate regions the average value can be taken as 0·004 with reasonable accuracy. Occasionally much higher values are reached; Callendar and McLeod record one as high as 0·323 over an 18-hour period when a large amount of cold water percolated in a short time. However, the average value is two to three times that due to pure thermal conduction, which indicates that the downward transfer of heat by percolation of water is considerable. This acts in opposition to the outward heat flow on which Kelvin based his famous estimate of 20 million years for the time since a solid crust first formed on the molten mass of the earth. Geologists objected to this estimate as much too low. The effect just discussed, and the discovery that radio-active rocks account in part for the observed temperature gradient in the earth, both necessitate an increase in Kelvin's estimate.

The Soil Atmosphere

Analysis of the air within the pore spaces of the soil shows that its composition is not very different from atmospheric air. It is somewhat higher in carbon-dioxide and lower in oxygen. The main biological activities in the soil cause the absorption of oxygen and evolution of carbon-dioxide, and there must be a constant exchange of these gases between soil and atmospheric air, otherwise the compositions would not be so similar. It has been calculated that in a normal fertile soil, the concentration of carbon-dioxide at the 20 cm. depth would be increased ten-fold in 14 hours if the gas did not escape.

The mechanism of the gaseous exchange has been the subject of some dispute. The factors concerned are those meteorological conditions causing bulk movement or streaming of air, and the inherent property of gases—diffusion. The earlier workers attributed the main importance to the meteorological conditions, which were thought sufficient in the aggregate to effect a complete rinsing of the soil air and replacement by atmospheric air. That some such action does take place is undeniable. Barometric changes cause expansion and contraction of the soil air; the daily temperature wave has the same effect; wind may force air in or suck it out of the soil; and rain will drive out some of the soil air. The difficulty is to place a numerical value on these factors. Romell,[6] in Sweden, has made a close examination of the problem. From measurements of carbon-dioxide production in the field he concluded that seven litres were produced daily per square metre of surface, and that to maintain the observed low concentration in the soil air, the latter has to be renewed to a depth of 20 cm. about once every hour. He defines this rate as " normal aeration " and then proceeds to examine the efficiency of the meteorological factors on that basis.

It is found that barometric and temperature changes, wind and rain produce only small effects. The effect of barometric changes is easily ascertained. The depth of penetration of atmospheric air is directly proportional to the depth of porous soil and to the change in pressure. If we take the normal daily barometric fluctuation as high as 4 mm. and assume that the depth of soil is 15 metres (both abnormally high values) the depth of rinsing is only 8 cm. and the time occupied is 12 hours. The effect of the temperature wave is much more difficult to calculate, because not only are different layers at different temperatures, but the soil as a whole has a different temperature from the air. Romell simplified the problem by calculating the movement of air from a cylinder of warm soil suspended in cooler air. Using extreme values throughout, it was found that only one-eighth of the normal aeration could be accounted for.

With regard to wind, the effect is to create an increased pressure on the windward side and a reduced pressure on the leeward side of any obstruction, and under the influence of this pressure gradient air may flow through the soil. Here, again, no precise estimate can be made, but the effect seems to be only a small fraction of the normal aeration requirements.

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[6] Romell. *Medd. f. Stat. Skogsforsoks*, **19**, 1922, pp. 125-359.

The effect of rainfall is easily assessed. The pore space in a normal soil rarely exceeds 40 per cent, and on the average about three-quarters is occupied by water and the remaining one-quarter (10 per cent of the total soil volume) is occupied by air. Thus an annual precipitation of, say, 100 cm. would displace the air over a 1,000 cm. depth, or a fifty-fold replacement of the conventional 20 cm. depth during the year, which is an insignificant contribution to the normal aeration requirements.

It appears, therefore, that none of the meteorological elements considered are, individually, adequate to explain the observed facts. Further, they are intermittent and even when acting together could only produce normal aeration conditions occasionally. Evidently, some other continuously operating factor is mainly responsible, and this points clearly to the phenomenon of gaseous diffusion.

Experiment and theory both show that diffusion is adequate to provide the normal aeration requirement which corresponds to an escape of 7 litres of carbon-dioxide per day per square metre of surface at 15°C. It is probable that the earlier workers dismissed diffusion in favour of the meteorological factors because the concentration of carbon-dioxide in the soil air was not much greater than in the atmosphere, and they therefore assumed that diffusion would have little effect. This was a mistake, for the theory of diffusion shows that although the actual volume of gas which diffuses is proportional to the concentration gradient, the velocity of diffusion is independent of this gradient, and it is the velocity which controls the degree of aeration.

A further point of practical importance is that the speed of diffusion, while depending on the pore space, is relatively unaffected by the actual dimensions of the pores. In other words, heavy or close-textured soils are not necessarily badly aerated. Most text books on agriculture and certainly all gardening books correctly lay great stress on adequate cultivation, but their implied explanation that this is essential to provide good "soil aeration" is not altogether correct.

Soil Moisture

In discussing soil temperature and aeration incidental references were made to soil moisture, which we must now consider in more detail. To the physicist, at any rate, the relations between the soil and its moisture content are perhaps the most interesting branch of the whole subject, while its practical aspects make it certainly the most important.

In the physical laboratory it is necessary to study the moisture relationships over a much wider range than those experienced in field conditions, where the moisture content varies from nothing to a maximum of about 40 per cent by volume for a waterlogged soil. Soil whose moisture content approaches the saturation figure may exist either in the porous state as in the field, or it may be worked by hand into a plastic condition resembling modelling clay. If further water be well mixed with the plastic mass, a paste is obtained which has both solid and liquid properties. In this stage the water is about 70 per cent of the total mass on a volume basis. Finally, with further dilution a suspension is obtained in which the moisture is present in great excess, and the suspension can display the phenomena of flocculation, cataphoresis, Brownian motion, etc., characteristic of colloidal material. In the paste and suspension ranges the clay, which is the name given to the smallest soil particles, is of most importance. Our knowledge of the physical properties of soil particles has been greatly extended by studying the paste and suspension ranges, but within the time limit of this lecture it is impossible to go into that phase of the subject. Those interested will find a full discussion in my recent book.[7]

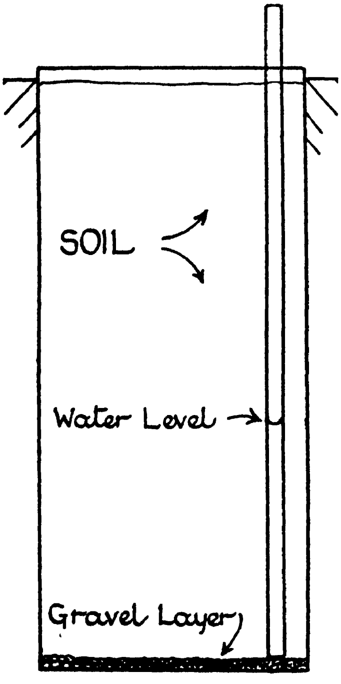

FIG. 6.—Soil cylinder for water-level measurements.

Soil being a porous material it was natural that the early experimenters should consider that water distribution and movement in it could be treated on the assumption that the pore spaces were essentially capillary tubes running through the mass. Of course, these tubes were irregular in width, length, and direction, but it appeared that this could be met by using a generalised form of the familiar capillary-tube formula, and in any case only the upward movement of water due to evaporation at the soil surface and the downward movement due to percolation seemed to be of importance. Interest centred in the upward capillary movement because of its practical bearing on the return to the upper soil layers of moisture in the lower depths of soil. The capillary-tube formula in which the height of rise (h) is inversely proportional to the tube radius (r) implied large values of h for soil owing to the small diameter of the average pore spaces. Estimates of many feet, even hundreds of feet, were not uncommon and they had considerable influence on the explanations of many cultivation operations. Thus, mulching was assumed to conserve soil moisture because the loose dry surface layer of soil broke capillary connection between the sub-soil water and the surface and thus checked the evaporation. Again, rolling the soil on which seedling plants were growing pressed the particles more closely together and thus increased capillary forces which brought extra water to the roots of the plants.

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[7] Keen: The physical properties of the soil (Longmans, 1931).

But when experiments are made on the capillary rise in soil the values obtained are disappointing for the capillary-tube hypotheses. A total rise of 4ft. is rarely obtained and the last stages of the ascent are so slow that but little water would become available to plant roots in a reasonable time.

The results of some Rothamsted experiments are of much interest in this connection because they extended over the great drought of 1921 when the long continuance of evaporation conditions at the soil surface gave the maximum opportunity for the so-called capillary rise of water from below. Cylinders about 2ft. 6in. in diameter and 6ft. deep were sunk into the soil to ground level and then carefully filled with soil. Provision was made for reading the level of the ground water in the cylinders. (Fig. 6). The soil was allowed to settle for several years before the experimental readings were begun. In the winter the soil became waterlogged to the surface. In the spring as evaporation increased, the level of the ground water began to fall. Rain checked or reversed this fall from time to time, but by confining attention to periods without rain, a composite curve could be built up showing how the water level would fall if evaporation at the surface were continued indefinitely.

The results for Rothamsted soil and a coarse and a fine sand are shown in Fig. 7, which provides a striking comment on the capillary-tube hypothesis of moisture rise. For, in spite of the long continued drought and relatively intense evaporation conditions at the surface, once the ground-water level had receded to about 75 cm. below the surface, the subsequent fall was so slow as to be almost negligible and was almost certainly due mainly to diffusion of water vapour from within the soil. Evidently, water is unable to rise in soil to anything like the height forecast by the capillary-tube hypothesis.

There was also a second difficulty. The theory required that the soil should be saturated between the ground-water level and the upper limit of the capillary rise, and this is not the case in practice. By reference to the irregular shape and size of the capillaries a qualitative explanation was given. An enlargement of the pore at some point might prevent any further rise of water there, while elsewhere the pores might be finer and still allow water to ascend. Hence, taking a cross-section of the soil, the water content would diminish with height, as is observed in practice.

These difficulties, and the fact that the attention of some mathematical physicists had been attracted to the problem led to an attempt to develop a theory of water movement on the analogy of heat or electrical flow. On this analogy the quantity of water (Q) flowing through a soil would, like the quantity of heat or electricity flowing in a conductor be dependent on two functions, the conductivity (K) and the potential gradient (e). Taking the simplest case

$$Q = -K\delta e/\delta x.$$

But the analogy is only formal because neither K nor e are constants as required by the theory, but depend on the moisture content there. The value of the conductivity (or the " capillary conductivity ") will depend in some measure on the thickness and distribution of the actual water film, and the " capillary potential " which is the attraction between the water and the soil is again a function of moisture content. Hence, further development of the theory necessitates development of K and e as functions of the moisture content, which is beset by grave mathematical and experimental difficulties. Courageous efforts have been made which I have no time to enter into here. But whether we are attracted or not to the mathematical development just mentioned, it is evidently necessary to have some definite idea of the physical relationship between the soil and its moisture content. We are forced back, therefore, to an examination of the geometry of the pore space in soil and a closer specification of the configuration of the water films in that pore space.

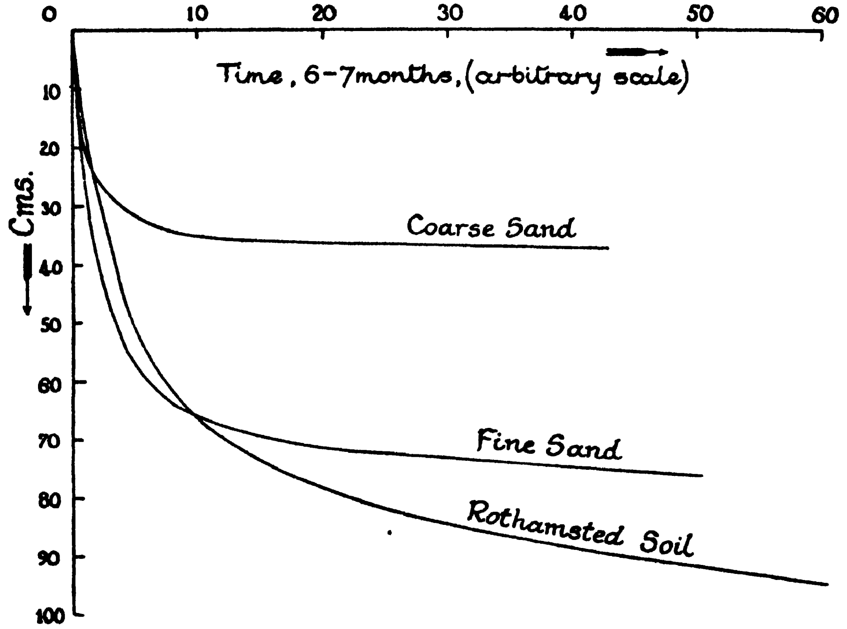

Fig. 7.—Downward movement of water level in initially saturated soil due to evaporation at surface.

(*From the " Proceedings and Papers of the First International Congress of Soil Science," Vol. 1.*)

The theoretical problem can be simplified by taking the " ideal " soil—one composed of spheres all of the same diameter and arranged in a systematic packing. The practical or experimental aspects can also be simplified, for the ideal soil actually exists. The " glistening dew " very popular a few years ago for picture postcards and Christmas cards is composed of minute glass spheres of very regular diameter. This material has been used with success in experimental work.

It is interesting to note that as long ago as 1890 Briggs,[8] in America, nearly solved the problem of moisture distribution. He pictured an ideal soil and pointed out that the water would tend to concentrate around the points of contact and that water movement could take place under the resultant influence of gravity and of surface tension over surfaces of varying curvatures. His work was an application to soils of the classical work on soap films by Reinold and Rucker. But one apparently minor, yet vital, step was omitted from his chain of reasoning, and the correct version was given by Haines[9] over 30 years later. Haines used the geometry of the ideal soil to characterise the pore space, and the pore space in turn specified the configuration of the water films. Briggs left out the intermediate step—the pore-space configuration—and referred the water film directly to the soil particles.

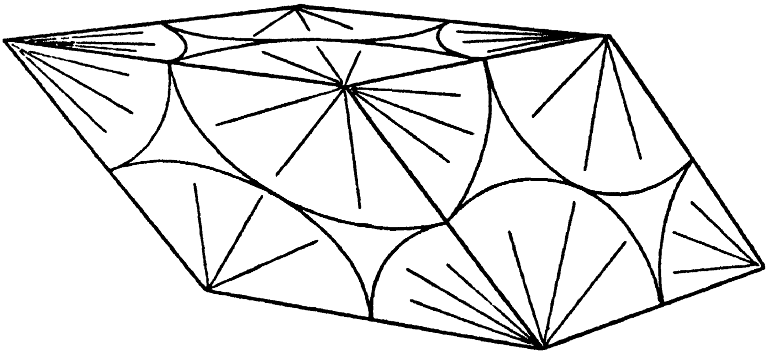

FIG. 8.—Unit element of spheres in closest packing.
(*From the " 19th Annual Report of the U.S. Geological Survey."*)

I do not intend to go into the details of the pore space in an ideal soil, but only to give its main features. If we take the pyramidal (or closest) packing typified by the familiar pile of cannon balls, we obtain an assemblage whose unit is a rhombohedron of side equal to the diameter of the sphere and with face angles 60° and 120° (Fig. 8). The pore space in this unit cell is shown in Fig. 9, which is taken from a plaster cast of the interior of the unit cell. It shows the cell-like nature of the pore space, which is, in fact, composed of two sets of regularly shaped spaces that may be called tetrahedral and rhomboidal cells. Each of the eight apices of a rhomboidal cell communicates with the apex of a tetrahedral cell. The latter has four apices, so there are twice as many tetrahedral cells as rhomboidal ones.

We therefore get a general picture of the pore space as composed of regularly shaped cells of two kinds communicating with

[8] Briggs. *Washington, D.C., Bull. U.S. Dept. Agric. Bur. Soils*, No. 10, 1897.
[9] Haines. *J. Agric. Sci.*, **20**, 1930, pp. 97-116.

each other through narrow necks. The next problem is, how is the water distributed within the cell-shaped pore space?

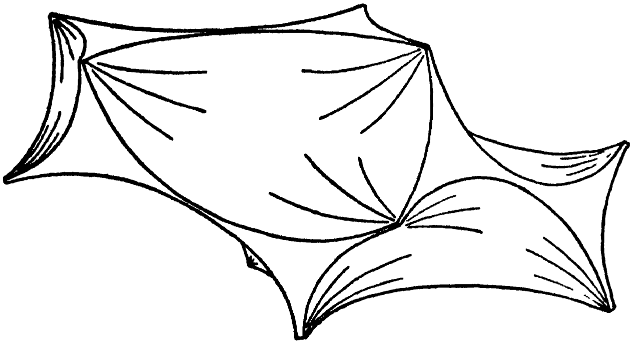

Fig. 9.—Shape of the pore space in unit element of spheres in closest packing. (*From the "19th Annual Report of the U.S. Geological Survey."*)

Starting from dryness, and assuming symmetrical distribution, the moisture would be found largely concentrated in annular rings around the points of contact of the spheres. Taking firstly a single water wedge (Fig. 10), we can consider with sufficient accuracy that it is an anchor-ring surface. Hence the well-known relation $p = T\,(1/c - 1/b)$ applies, where p is the deficit of pressure under the curved water surface and T is the surface tension. Both c and b can be expressed in terms of r the radius of the sphere, hence the pressure deficiency under the curved surface is equal to T/r multiplied by a numerical factor. Eventually the water rings will grow to a size such that they come into contact. This occurs first in the four planes forming the centres of the four spheres enclosing the tetrahedral cell (Fig. 11) when the moisture content is about 3·55 per cent by weight. At this stage the general distribution is shown by Fig. 12, in which the points of contact of the spheres are shown by crosses. The pressure deficiency which has been decreasing in value is found by calculation to be $p = 4{\cdot}1\ T/r$. The pore space not filled by water can be pictured as "rounded" rhomboidal and tetrahedral cells bounded by the surface of the water films as shown in Fig. 13. Simple considerations of surface tension show that there is a tendency for the films within the cells to collapse, and this tendency is balanced by the hour-glass-shaped waists at the communicating corners which supply the balancing outward tension. As the

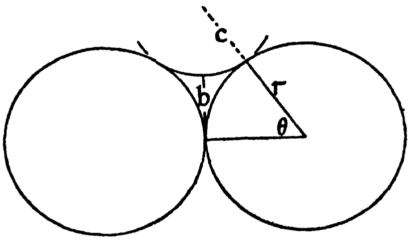

Fig. 10.—The water ring between two spheres.

moisture content is further increased the film at the waists thickens, and to maintain the same total curvature, the curvature in the other directions at right angles must also change. The water film bulges inwards, the air channel becomes restricted and ultimately the waist closes and becomes filled with water. The new film shape is shown by the thick lines at A in Fig. 13, the space between them being filled with water. This changes the general shape of the water film within the cells into one approximating to the true bubble form. The film curvatures are additive so the new film form has a higher total curvature and therefore a higher

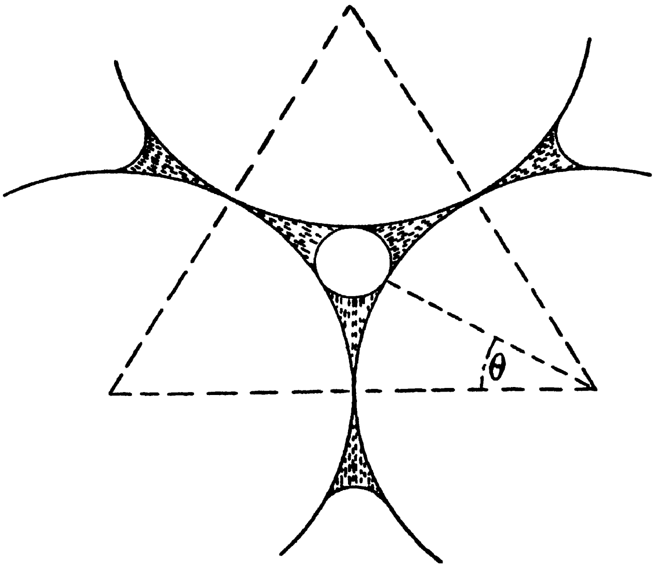

FIG 11.—The water rings around points of contact of three spheres
(*From the "Journal of Agricultural Science"*)

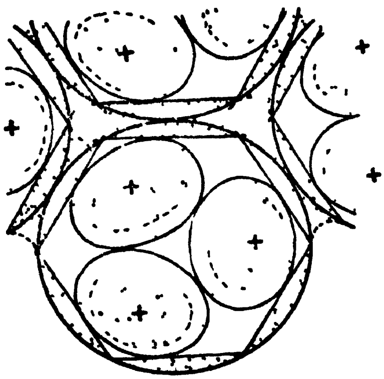

FIG 12.—Distribution of water at first stage of coalescence.

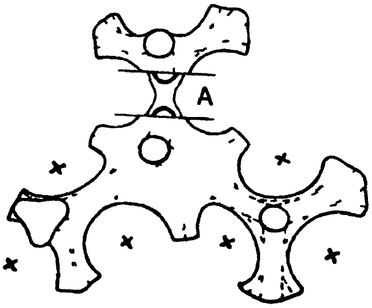

FIG. 13.—Perspective of continuous air space at first stage of coalescence. Development of final stage shown at one neck.
(*From the "Journal of Agricultural Science."*

pressure deficiency. The approximate value is 6·9 T/r. Eventually, as more waists collapse, the cell bubble becomes more unstable and itself collapses; this is a kind of " trigger " action affecting neighbouring cells which also fill with water, possibly at the expense of neighbouring parts of the soil, thus affecting a general redistribution of the water. The energy for this movement is found by the decrease in surface area of the water film that accompanies the closing of the cells by water. As further water is added the zone of saturation extends by similar and relatively sudden saturation of the cells with very little change in the value of the pressure deficiency. Ultimately the soil becomes saturated, and the pressure deficiency falls rapidly as the menisci in the external pores of the mass disappear until the soil is no longer saturated, but flooded, and its coherence disappears.

If now we follow the reverse change from saturation to dryness, the first stage is the formation of menisci in the surface pores, which advance inward. When the meniscus has reached the narrowest part of the waist of the pore the pressure deficiency is, by simple calculation, about 12·9 T/r. As the meniscus proceeds further, the diameter of the pore expands so it is in an unstable condition and the entry into the cell takes place suddenly. The displaced water redistributes itself, the pressure deficiency meanwhile falling slightly. With reduction of moisture, the menisci which have formed in the exit pores of the first layer of cells repeat the process and eventually there is a thread-like series of air passages through the soil although the great bulk of the pore space is still occupied by water. Not all the waists are necessarily open in this stage, since for evacuation of a cell only one entry and one exit pore are necessary. With further reduction in moisture content more and more of the pores become opened until, ultimately, the remaining water is left in discrete rings around the points of contact. The size of one of these rings, when first formed, is smaller than the maximum size of the water rings in the ascending water-content stage of the cycle, for, as we have seen, the latter persist up to pressure-deficiency values of 4·1 T/r before the first stage of coalescence begins. Thus over the range 4·1 T/r to 12·9 T/r different shapes of the water film are possible at the same general value of the pressure deficiency and can exist side by side in equilibrium. In other words, there is a hysteresis cycle in the moisture content. Haines[10] has verified this experimentally both for the ideal soil and for sand. His results are shown in Fig. 14; they illustrate the two points of immediate practical interest: firstly, that the distribution of water within the soil is not necessarily uniform from point to point, and secondly, these different moisture contents can be in equilibrium with each other. Thus, just as the phrase the " temperature of the soil " has no exact meaning, the " moisture content of the soil " is not necessarily unique, but an average quantity. Adjacent portions of the soil may have moisture contents differing but little from the average value; on the other hand they may not, and the extent of the differences will depend on the manner in which the particular average value has been approached. It is of much interest to note that the curves of Fig. 14 also give the equilibrium distri-

[10] Haines. loc. cit.

bution of water from the soil surface down to the saturation or ground-water level. The pressure-deficiency values in this case are replaced by the heights that give the same gravitational head, and then the falling-moisture (right-hand) curve refers to a saturated soil allowed to drain, while the rising-moisture (left-hand) curve applies to the equilibrium values when a dry soil column is placed with its base in water. Each curve shows the decrease in moisture with height, and is thus in accord with field observations.

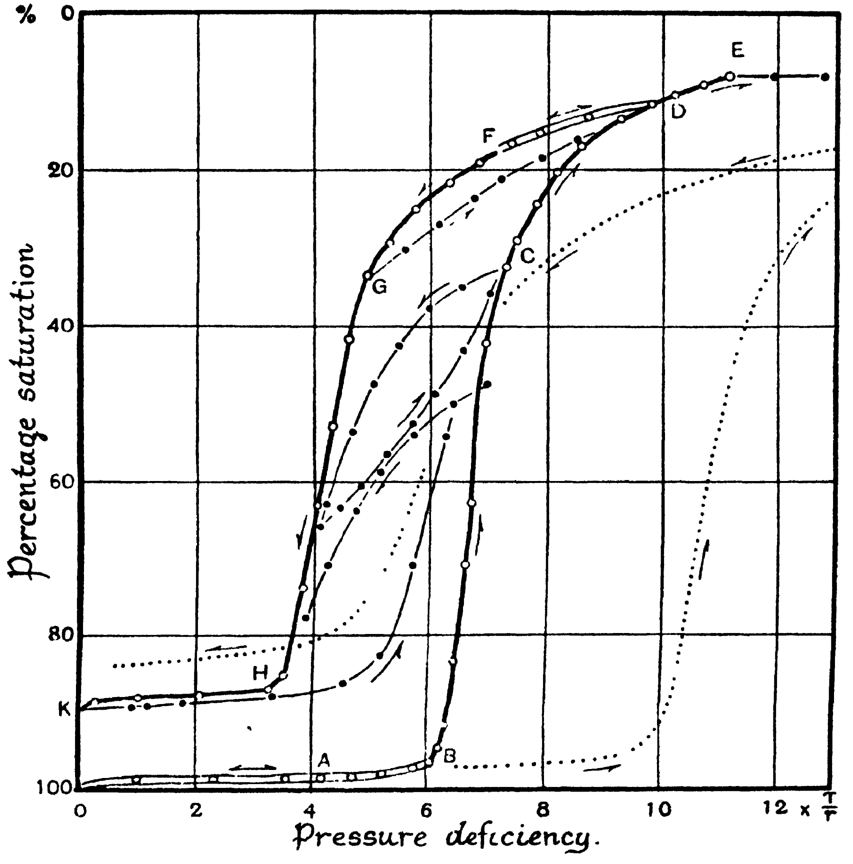

FIG. 14.—Relation between percentage saturation of pore space and pressure deficiency for "glistening dew," and sand (dotted curve)

(*From the "Journal of Agricultural Science."*)

SOIL CULTIVATION

The main purpose of cultivation is to provide the best conditions for the growth of a given plant or crop.

One obvious function is to kill weeds, which otherwise compete for food and water with the cultivated plants. The bulk of the operations are directed towards altering the physical condition of the soil either by mechanical manipulation so that weathering influences may work more effectively, or by changing the degree of packing and sub-division of the surface layer in the attempt to control soil moisture.

Weathering influences are most important during the autumn and winter and on heavy soil it is very difficult to get a tilth in the spring if the winter has been wet and open. Experiments at Rothamsted show that in a season of this type the most efficient cultivation implement does not produce so good a tilth as the least efficient implement after a good winter. The measurements were made by taking blocks of the soil immediately before and after cultivation and passing them gently over a series of sieves of graduated mesh size so that the soil was divided into a number of fractions; the largest lumps being those that failed to pass the 1½-inch mesh and the smallest being those that passed a 0·1-inch mesh. Comparisons of the two sets of figures before and after the cultivation give a measure of the degree of soil comminution effected by the implement.

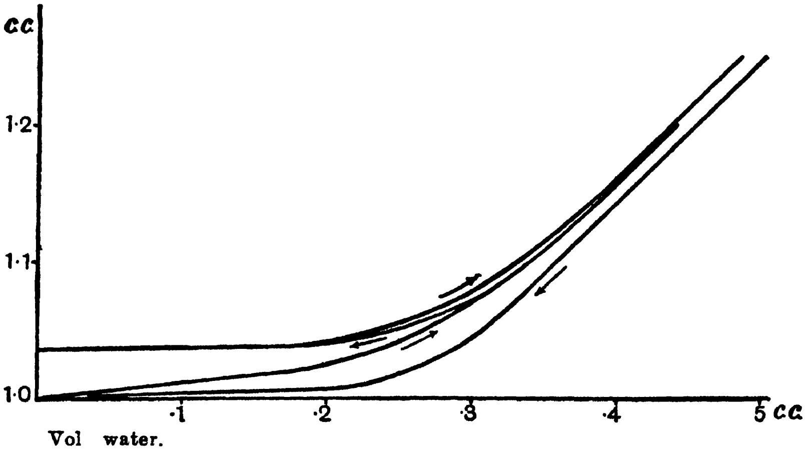

FIG. 15.—Effect of alternations of drying and wetting on volume of a soil block.
(*From the "Journal of Agricultural Science"*)

The effect of the alternations of weather—wetness and dryness, frost and thaw—causes the furrow slice to break up into smaller pieces, along lines of weakness. Even a plastic mass of clay worked up by hand with water into the condition of modelling clay with the bulk of the air excluded, will crumble after one or two dryings and remoistenings.[11] The course of this process is illustrated in Fig. 15. It will be observed that the first stages of drying result in a loss of 1 c.c. of volume for each c.c. of water lost. Then the shrinkage slows up as the soil particles come into actual solid contact with each other; at this stage air is entering the pores of the mass. On careful remoistening the block expands again, but its volume at a given moisture content has increased. This increase is not primarily due to a swelling of the soil material as such, but is the expression of an increase in the pore space under the stresses produced by the volume changes. The increases are per-

[11] Haines. *J. Agric. Sci.*, **13**, 1923, pp. 296-310.

manent and after one or two further alternations the block is so fragile that it crumbles to pieces.

The operations subsequent to ploughing are largely concerned with stirring the surface, and it is generally assumed by agriculturists that this conserves soil moisture, partly by reducing the soil temperature by a dry mulch of low heat conductivity, but mainly by preventing the capillary rise of moisture to the actual soil surface. As we have seen the latter effect is doubtful, at any rate the explanation is probably not correct. The evidence is, however, somewhat conflicting; on balance it is definitely against this hypothesis, and it seems that mulching does not, in general, conserve soil moisture unless there is actually a ground-water table within some 6 feet from the surface. That it has an agricultural value is undeniable, but much of this must be ascribed to the simple effect of destroying seedling weeds as they appear and thus reducing their competition with cultivated crops for food and moisture. A second effect, which is especially important on medium and heavy soils, is that the tendency of the surface layer is to form a hard crust or " cap " when the soil dries out. This layer is liable to cause injury to the roots of young plants; cultivation prevents the formation of the crust.

A converse effect to mulching is the consolidation of the soil surface by rolling. The conventional explanation is that the reduction in pore space encourages capillary rise of water and so brings moisture more abundantly to the roots of the young plants. Here, again, the beneficial effect of rolling is undeniable, but the explanation is wrong. We have seen how slow the movement of water is in field soils when the water content has fallen below a certain value. On the other hand, if the soil contained enough water for the capillary movement to be enhanced by reducing the pore spaces then, in that condition, rolling would have disastrous effects on the tilth. The real effect is largely a mechanical one; it gives the plant a firmer hold of the soil by remedying the loosening action of weather alternations on soil structure, and as it presses the soil more closely around the roots, they are naturally able to obtain the water more readily, and in addition the compression increases the amount of water per unit volume of the soil.

The physical laws controlling the distribution of the water film in soil, outlined in the preceding section, show that when the moisture content is reduced at one place, movement to that region from adjacent parts does not necessarily occur at once. There is a tendency to regard the plant root as relatively static and to consider that the water moves through the soil by capillary pores towards the root under the influence of the moisture gradient established by the withdrawal of water by the root itself. While this effect certainly occurs, it is desirable to remember that such movements are slow. In the active stages of plant growth the root itself grows out in search of moisture.

[The lecture concluded with an account of the historical development of the plough, illustrated by lantern-slide copies of tapestries, illuminated manuscripts, etc.]

Permission to reproduce the illustrations has kindly been given by the various authorities quoted under each diagram. Thanks are

also due to Messrs. Longmans, Green and Co., Ltd., for the loan of the blocks which all appear in " The Physical Properties of the Soil," B. A. Keen, 1931.

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THE EFFECT OF CLIMATIC VARIATIONS ON THE PLASTICITY OF SOIL.

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INTRODUCTION.

FIELD experiments on different methods of soil cultivation carried out at Rothamsted (1) have shown that for spring seed-bed production the effect of the implements used is subsidiary to the effect of the climatic conditions of the previous winter. The ease (or difficulty) with which the soil breaks up must be related to the various plasticity phenomena which are being investigated at Rothamsted (2–13). It therefore seemed desirable to make a preliminary study of the effect of meteorological changes on the plastic properties of worked and unworked soil, especially as Vinokurof (14) has already shown that the quantity of highly dispersed soil particles (which plays an important part in plasticity phenomena) is at a minimum when both rainfall and temperature are highest, *i.e.* in the Russian summer.

THE PLASTIC CONSTANTS.

As the full details of the measurements will be found in the papers referred to (2–9), it is only necessary to give brief definitions of the constants employed in the present investigation.

Plasticity has been defined as "That property which enables a material to be deformed continuously and permanently without rupture during the application of a force which exceeds the yield value of the material" (15), and the word "plasticity" itself will be reserved to describe the extent to which such a material can be so deformed.

Plasticity itself is difficult to measure directly in the case of a soil, but a test has been devised in which a paste made by mixing the soil with water is forced through a narrow tube under pressure; and from the curve relating rate-of-flow to shearing stress, an empirical constant (B') can be determined which has been shown to be closely related to plasticity (5, 9). This constant is known as the flow-plasticity.

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A full description of the phenomena observed when a soil-paste is forced through a capillary tube has been given in a previous paper in this journal (3). It was found that at all stresses below a certain critical stress (A), there is no movement of the paste in the capillary. When the stress exceeds A, the paste flows as a solid plug through the tube, and at a still higher well-defined stress (B) a type of streamline flow starts to take place near the wall of the tube, and the rate of flow increases sharply. The values of A and B both increase with increasing dry-matter content of the paste, and if the A values for several dry-matter contents are plotted against the B values, the A/B curve is found to be linear, usually making (on extrapolation) a small intercept on the B-axis (the B intercept). The value of B when A is 1 dyne/mm.^{2} is defined as the flow-plasticity (B').

In the case of soil, there are two other plastic properties which are important, (*a*) the dry-matter content of the soil for a given state of consistency, and (*b*) the force required to cause a deformation (*i.e.* the yield value in the definition of plasticity given above). With a view to investigating the property (*a*), two further constants were defined; namely the dry-matter content of a paste of which the value of A would be 1 dyne/mm.^{2} (K'), and the dry-matter content corresponding to a value of B equal to 10 dynes/mm.^{2} (K_B). Since either K' or K_B can be taken as a rough measure of consistency, it is clear that an increase in K' or K_B corresponds to an increase in the dry-matter content of the soil for a given consistency. Property (*b*) can be measured for pastes in the plastometer as already described in earlier papers (2–7), and for the soil itself at approximately field-moisture content in the pachimeter (10–13), but since any changes which may occur in this property under seasonal climatic variations are certainly small, it is not included among the properties studied in the present work. The experiments to be described consist of a study of the way in which B', K', K_B and the B-intercept vary with the dry-matter content (K_s) of the soil in the field from which the pastes were prepared, with the soil temperature (θ), and with the rate of change of these properties with time. These factors are studied for natural as well as for simply "cultivated" soils. It will be seen from the discussion below that in spite of the drastic dispersion and sieving involved in preparing the soil pastes, the plastic constants are definitely dependent on the previous moisture and temperature history of the soil.

THE SOIL INVESTIGATED.

A mixture of Rothamsted and Woburn soil, giving convenient flow-plasticity figures, was thoroughly mixed and pounded in the air-dry state (a mechanical analysis is given in Table I).

Table I. *Mechanical analysis of soil.*

Fraction	Oven-dry basis	Ignited basis
Coarse sand	23·4	23·1
Fine sand	28·2	28·1
Silt	8·5	8·7
Clay	31·0	26·5
Air-dry moisture	5·4	5·4
Carbonates	—	—
Loss on solution	0·8	0·8
Loss on ignition	—	7·1
Difference	+2·7	+0·3
	100·0	100·0

The soil was then sieved (3 mm.) and tipped into the container. The container consisted of a breeze-block box of internal dimensions 2 ft. × 2 ft. × 4 in. and thickness of sides about 2 in., which was buried with the top flush with the level of the ground (in the open), in the meteorological enclosure at Rothamsted. Soil samples were removed from this by means of a cylinder (diameter 1·1 in.) pushed down on to the bottom of the container. The hole so formed was immediately filled up with kaolin weathered under the same conditions, and kept in a neighbouring hole. The sample of moist soil was placed in a corked bottle, removed to the laboratory, a sample taken to determine K_s, and the rest was worked at once into a paste with distilled water, forced through a 100 mesh per in. sieve, thoroughly stirred, and then tested in the plastometer in the usual way(2). The soil in the container was thoroughly weathered before any samples were taken, and was kept carefully weeded. Owing to two rather bad infestations by ants, which could not be removed by smoke, the soil was twice sprinkled lightly with tetrachlorethane ($C_2H_2Cl_4$). This eliminated the ants, but it is uncertain whether it had any temporary effect on the soil. Careful laboratory experiments failed to show any change in the physical properties of laboratory soil samples treated in this way.

Measurements were taken every few days, records being kept of K_s, K', K_B, B', and B intercepts. The temperature (θ) of a neighbouring sample of soil (4 in. depth bare soil) is recorded daily in the meteorological records of the Station, and these values were also noted for comparison.

The experiment was started in January, 1931, and continued until the end of September. On April 13 part of the soil was flattened by means of a flat 1 stone weight, but, since this seemed to have little effect on the constants, the same part was afterwards lightly dug up with a trowel (June 21). Observations were taken on both the worked and unworked areas.

The results of the measurements are given in Table II.

Table II.

No.	Date (1931)	B intercept	K_s	B'	K'	K_B	θ°
(*a*) Untreated soil							
1	27. i	0	74·8	6·6	37·4 (?)	39·5 (?)	34
2	7. ii	0	73·3	6·0	40 8	42·6	34
3	16. ii	0	74·0	6·3	39·1	40·7	37
4	26. ii	0·2	75·4	4·8	37·1	40·3	45
5	9. iii	1·1	73·6	8·4	40·4	40·8	31
6	20. iii	0	80·2	6·9	38·6	40·0	44
7	1. iv	0·2	83·3	9·6	40·6	40·8	38
8	13. iv	0	79·6	5·1	36·8	40·4	45
9	23. iv	0·6	75·8	7·3	40·2	41·8	43
10	4. v	0	74·6	4·5	39·5	43·0	45
11	14. v	0·8	81·8	7·0	41·0	42·5	56
12	26. v	0	75·4	4·3	38·3	42·4 (?)	61
13	6. vi	0	76·5	5·2	39·6	42·0	59
14	17. vi	1·5	81·4	4·6	38·7	42·3	61
15	29. vi	0	82·8	6·5	39·4	41·4	65
16	9. vii	0	86·4	5·4	39·6	44·8 (?)	62
17	21. vii	0·3	76·7	5·0	41·7	44·3 (?)	56
18	31. vii	0·6	77·5	4·9	42·7	45·3	61
19	11. viii	0·8	73·6	4·5	40·9	43·6 (?)	55
20	22. viii	0·6	75·6	5·5	41·3	49·2	57
21	25. viii	0	75·4	5·7	39·6 (?)	41·5 (?)	53
22	7. ix	0	70·9	4·7	46·7 (?)	51·2 (?)	49
23	18. ix	0·6	76·7	4·5	53·7 (?)	54·4 (?)	57
24	24. ix	1·0	76·0	6·7	42·3	43·8	52
25	30. ix	0·2	80·2	9·1	44·5	44·8	54
(*b*) Flattened soil							
1	17. iv	0	77·5	6·8	39·1	42·0	45
2	28. iv	0	75·6	5·1	38·1	41·1	44
3	8. v	0 (?)	77·6	7·7	41·1	42·2	52
4	19. v	0·5	75·8	4·5	39·6	42·3	49
5	30. v	0·3	78·6	6·3	40·6	43·4	59
6	11. vi	0·4	77·5	4·3	38·0	41·8	59
7	22. vi	0·5	79·8	5·2	39·1	41·6	62
(*c*) Dug soil							
1	3. vii	0	84·0	4·4	39·2	41·8	63
2	15. vii	0·5 (?)	76·1	4·4	42·8	45·3	59
3	27. vii	0·2 (?)	75·8	4·7	43·8	46·5	60
4	7. viii	0·8	77·8	4·9	41·3	43·7	63
5	21. viii	0·6	72·9	6·2	40·2	43·5	57
6	1. ix	0	65·8	4·0 (?)	43·7 (?)	46·2 (?)	58
7	14. ix	0	78·2	5·5 (?)	50·8 (?)	53·3 (?)	50
8	23. ix	0	75·0	5·6	40·3	42·8	52
9	28. ix	0	75·9	4·8	39·6	42·4	51

All plastometric measurements were made at 25° C.
Dry-matter contents are given as percentage dry matter on total weight of paste.

DISCUSSION OF RESULTS.

The data of Table II have been statistically analysed, and the following conclusions have been drawn:

1. In the case of "untreated" soil, the flow-plasticity (B') is significantly lower for high temperatures and for wet soil, and higher in cold, dry weather. It thus varies in the same sense as the quantity of highly dispersed material measured by Vinokurof. The connection between the measure of plasticity (B'), the temperature (θ), and the dry-matter content of the soil (K_s), was investigated by fitting regressions. The data for the untreated, flattened, and dug soils were analysed separately, linear regressions of the form $B' = \bar{B}' + b_\theta (\theta - \bar{\theta}) + b_K (K_s - \bar{K}_s)$ being fitted. The results were as follows:

Table III.

Soil	Observations	B'	$\bar{\theta}$	K_s	b_θ	Standard deviation	b_K	Standard deviation
Untreated	25	5·964	49·84	77·26	−0·099	±0·025	+0·226	±0·067
Flattened		5·700	52·43	77·49	−0·193	±0·100	+1·032	±0·516
Dug	9	4·944	57·22	75·72	−0·063	±0·052	+0·022	±0·053

The values of b_θ and b_K for untreated soil are certainly significant. The values for the flattened and dug soils are not significant, as might be expected, since the number of observations is much smaller in the last two cases. More important, however, is the fact that they are not significantly different from the values for untreated soil. From the regression formula for untreated soil, we may compute the expected mean values of B' for the flattened and dug soil. For the flattened soil, the expected value is 5·75 against the actual value of 5·70, and for the dug soil, the expected value is 4·88 against the actual value of 4·94. Since the standard deviation of $\bar{B}'$ is 0·22, the agreement, which is also affected by errors in b_θ and b_K, is even closer than we have a right to expect on the assumption that flattening and digging produce no effect. This tends to confirm the general hypothesis that weather is far more effective than cultivation in modifying the physical properties of the soil.

2. The statistical analysis shows that although the linear regression on θ and K_s accounts for a good deal of the variation of B', there is still a considerable residue, and it is of interest to inquire whether the rate at which θ and K_s were changing may not have had some important effect on flow-plasticity. Unfortunately, the very scanty knowledge available about the way in which θ and K_s were changing made it impossible to show any significant connection for the experiments described. It is felt that such a connection may well exist, but a carefully controlled

the mass of soil with water, and then forcing it through a fine sieve, the flow plasticity of the paste still depends on the climatic history of the soil from which it is prepared.

2. The soil has, in general, a higher plasticity in cold and dry weather and a lower plasticity in warm and wet weather. These results seem to confirm the conclusions of Vinokurof about seasonal fluctuations in the quantity of highly dispersed particles.

3. Data obtained were inadequate to show whether the plastic constants were functions of the rates of change of moisture and temperature with time.

4. The concentrations of soil in pastes having certain standard consistency constants were determined, but were not found to vary regularly with temperature or moisture.

5. Comparisons were made on untreated, flattened, and dug soils, but no regular effect could be shown to have been produced by flattening or digging. The differences in the means of certain constants are well explained by the fact that each treatment was studied at a separate time (the tests on untreated soil running throughout the whole course of the experiment), and there were seasonal fluctuations in some of the properties both in the untreated, and in the worked soils.

6. The possible significance of these results is discussed.

The authors wish to express their thanks to Dr R. K. Schofield, of the Physical Department, for his suggestions and help given in the experimental part of this work, and for the interest that he has taken throughout its course.

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THE MEASUREMENT OF ELECTRICAL CONDUCTIVITY OF AQUEOUS SOIL SUSPENSION AND ITS USE IN SOIL FERTILITY STUDIES[1].

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(With Five Text-figures.)

INTRODUCTION.

ATKINS (1), as a result of his determinations of electrical conductivity of aqueous extracts of soil after different periods of extraction, observed that there was a rapid increase in conductivity as the extraction was prolonged in the case of fertile soils, while there was little or no increase in the case of infertile soils. He suggested that the increase in conductivity with prolonged extraction was partly brought about by bacteria, and the method might serve to measure the inherent fertility of soils. Later, several workers (41, 12, 32) obtained promising results with the method, and the writer (30) showed that for Indian soils collected at the same time from adjacent plots the increase in conductivity of soil suspensions after 8–10 days' standing corresponded with the crop yields.

Utilisation of the method in investigations of fertility necessitated standardisation and tests under a wide range of climatic and other conditions. An extensive study of the technique was also desirable, since the measurement of conductivity has been often employed in other soil investigations (2, 3, 7, 19, 26, 27, 35).

A number of factors that might be expected to affect the conductivity are briefly discussed below:

(i) *The influence of air drying.* Air drying of soil greatly increases the amount of water-soluble inorganic (17, 20, 21) and organic (22) soil constituents. Rahn (29) observed that after air drying, the capacity for biological activity is increased by an amount varying from 60 per cent. in a garden soil to 10–30 per cent. in a light field soil. Further, Waksman and Starkey (39) showed that biological activity, as measured by the evolution of CO_2, was influenced by the duration of the air-drying period.

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[1] Part of the thesis presented to the University of London for the degree of Doctor of Philosophy.

(ii) *Seasonal changes.* Electrical conductivity of soil extract is different at different depths (19, 31) and tends to increase at the surface following a prolonged dry period owing to accumulation of soluble salts lifted from lower depths by capillarity (31). Wright (41) showed qualitatively that the conductivities during the dry season were higher than those during rainy weather. On the other hand, the studies of Cutler, Crump and Sandon (9) on changes in the micro-organic population have shown the complexity of this phase of the problem, which is taken up in detail below.

(iii) *The effect of crop growth.* It is now generally held that the soluble salt content of soil is decreased as crop growth proceeds. This has been shown by Stewart (34) who analysed 1 : 5 aqueous extract of soil; by Hoagland (18) who followed the soil solution concentration by the freezing-point depression method (5); by Stewart and Martin (35) who employed the electrical resistance of aqueous soil extract.

Microbiological studies by Starkey (33) and by Greaves and associates (16) indicate that the organisms are more abundant near plant roots, and are less abundant in fallow than cropped soils.

(iv) *The effect of manures.* The addition of any manure containing soluble compounds will evidently directly affect the electrical conductivity of aqueous extracts of soil. There will also be an indirect effect due to the resulting changes in the micro-organic population. Fred and Hart (13) found that addition of inorganic fertilisers, especially soluble phosphates, to soils causes an increase in the ammonification, CO_2 evolution, and total number of bacteria in soil. The increased CO_2 production favours the solution of insoluble minerals of the soil. Greaves and others (14, 15), however, pointed out that carbonates of sodium, potassium, calcium, magnesium, etc., are toxic and check bacterial activities in soil.

On the other hand, organic material and fertilisers stimulate the development of various groups of organisms (40). The presence of organic residues containing excess energy material leads to a temporarily marked increase in the micro-organisms and a proportional assimilation of plant food elements. However, as the excess energy material becomes exhausted an increased liberation of plant food takes place (10).

DEVELOPMENT OF THE EXPERIMENTAL METHOD.

The ratio of soil to water was 1 : 5 throughout this work. Change in this ratio is known to affect the conductivity (34), and it appeared desirable to use the generally accepted proportion 1 : 5.

The dip electrode was of the same type already used (30) except for minor modifications. The cell constant was determined every 3 weeks. All measurements of conductivity were made at 25° C. Only freshly boiled distilled water (conductivity 1–2 × 10^{-6} mhos) was used. The conductivities are multiplied by a million to give whole numbers and are expressed in "mhos" (reciprocal ohms) throughout.

Effect of air drying.

As expected (17, 20, 21) the conductivity of soils was found to increase generally on air drying, but this increase was very irregular for Rothamsted soil, being roughly 20–30 per cent. for unmanured and 30–40 per cent. for manured plots. In view of this it appeared advisable to confine attention to the conductivity of suspensions of soil brought fresh from the field.

Technique for moist soil.

Rapid determination of moisture. One of the difficulties in preparing suspensions of fresh soils from the field is to find out the exact amount for the 1 : 5 ratio. However, for Rothamsted soil it was observed that a variation from the correct amount by not more than 3 per cent. changed the conductivity very little, and that such changes were within sampling errors. Consequently in each case a rapid determination of the moisture content of the fresh soil[1] was made so that the calculated amount of soil never differed from that needed for the 1 : 5 ratio by more than 1·5 per cent.

Effect of shaking. The necessity for shaking a soil water mixture prior to a conductivity measurement has already been pointed out by the writer (30). Further evidence in support of this was obtained. It was observed that the conductivities at different depths in the supernatant liquid column of a suspension which had been allowed to stand for several hours were different, but when the suspension was shaken thoroughly an uniform conductivity was obtained at all depths which was very nearly equal to that of the semi-clear centrifuged extract.

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[1] Methods suggested by Bouyoucos (4) and Nitzch (26) which consist in estimating the moisture content from the change in the specific gravity of alcohol after adding it to the moist soil were found unsatisfactory in the case of heavy Rothamsted soils, giving results which differed often by more than 5 per cent. from the oven-dry figures. But direct drying over a rose burner gave results which seldom exceeded the oven-dry figures by 1 per cent. The procedure adopted was to place 10–15 gm. of moist soil in a silica basin over a rose burner. The soil was constantly stirred and small clods broken as drying proceeded with a thin metal rod. The flame was removed once or twice for about 20 sec. during the heating. The operation is completed in 3–6 min.

Furthermore, with moist Rothamsted soils, some of which contain 18–25 per cent. clay, the conductivity was found to vary with the degree of dispersion of the soil in the suspension. Thus of the suspensions of the following four samples of soil taken at random from an unmanured plot, those which were shaken in an end-over-end shaker for 1 hour prior to the measurement had conductivities agreeing more closely with each other (column B, Table I) than did the conductivities of the suspensions which were shaken by hand for a few minutes only (column A, Table I).

Table I.

	Conductivities of suspension	
Sample no.	A Shaken for a few min.	B Shaken for 1 hour
1	145	190
2	156	188
3	153	187
4	182	192
Mean	159	189
Standard deviation	16 or 10 %	2·2 or 1·2 %

In some cases, however, especially in the case of manured plots, conductivities of several samples collected on the same day from a plot were found to differ widely even when the suspensions were shaken for

Table II.

	Conductivity after shaking for							
Sample no.	1 hr.	4 hr.	1 day	2 days	3 days	4 days	6 days	7 days
1*a*	104	121	167	196	218	231	251	257
1*b*	105	121	165	196	220	—	—	—
Difference	1	0	2	0	2	—	—	—
2*a*	129	152	199	226	252	263	283	300
2*b*	122	146	197	231	260	—	—	—
Difference	7	6	2	5	8	—	—	—
3*a*	114	132	185	225	256	—	—	—
3*b*	108	127	175	213	240	—	—	—
Difference	6	5	10	12	16	—	—	—
Difference between 1*a* and 2*a*	25	31	32	30	34	32	32	43

1 hour. A longer period of shaking was tried with no consequent gain in the agreement. In Table II the results of a typical experiment are given. *a* and *b* represent duplicate suspensions of the same sample.

It is evident from the above table that the measurements for the two suspensions 1*a* and 2*a* (which were selected for shaking for 1 week since their conductivities after 1 hour's shaking were the highest and lowest in the whole set) maintained a steady difference of about 30×10^{-6} mhos throughout. Further, the differences between duplicate suspensions were also maintained throughout in each case. It is clear, therefore, that the maximum dispersion of the soil obtainable by shaking is attained after shaking the suspension for 1 hour. Consequently differences in the measurements such as those seen above are due to soil heterogeneity and unequal distribution of manure. However, fair agreement was obtained in the measurements for several composite samples, each a mixture of three separate holes, from such plots, the standard deviations being generally 2 per cent.

Sampling procedures. It will be found later that soil samples were brought periodically from certain Rothamsted classical and non-classical plots throughout the year for determination of the electrical conductivity. In general the procedure was to mark out an area 18 ft. by 12 ft. in each plot by means of wooden pegs whose positions were noted down with respect to certain fixed objects outside the plot so that they were replaced accurately whenever they were disturbed by field operations. The composite laboratory sample was obtained by mixing together the soil from three holes, 0–4 in., bored across the marked out area at 6 ft. apart. These samples were collected successively at 1 ft. apart from one end of the area at intervals of about 3 weeks until the other end 18 ft. away was reached. The procedure was then reversed after slightly changing the positions of the pegs.

Final method.

The composite sample was brought to the laboratory and whenever possible was sifted through a 3 mm. sieve without delay. The percentage of soil moisture was then quickly determined and a mixture having the ratio of 1 part of oven-dry soil to 5 parts of water was prepared from the moist soil with 100 c.c. conductivity water in a half-pint bottle. The mouth of the bottle was then closed by a paraffined cork and the mixture shaken for 1 hour in an end-over-end shaker rotating at 32–36 revolutions per minute. As temperature fluctuations have a marked effect on conductivity the bottle was then placed in the thermostat maintained at

25° C. for 40–45 min. The conductivity cell was thoroughly washed with ordinary and finally with freshly boiled distilled water. After water had been removed as far as possible by draining and by means of a filter paper (the conductivity was unaffected by the little water that still remained), the cell was placed in the suspension which had just been shaken thoroughly for a few seconds by hand and the electrical resistance was measured by means of a Wheatstone Bridge Box with non-inductive resistances, using a buzzer and a telephone. The cell was then withdrawn, and the suspension was once more shaken thoroughly by hand. The cell was replaced quickly and the balancing resistance was determined with as little delay as possible. Usually the second resistance was slightly lower in value than the first, but no further reduction took place if the cell was removed and inserted again after shaking the suspension for the third time. After this measurement, which will be referred to as "initial conductivity," had been made the bottle containing the suspension was tightly corked and was left in the thermostat. After four days the suspension was shaken for half a minute, and after seven days the final conductivity was measured. The difference between the final and the initial conductivities gave what will be referred to as "7 days' increase."

The above technique was found to be equally efficient for air-dry soils. Such measurements served as convenient checks on those for the corresponding fresh soils.

EXPERIMENTAL RESULTS AND DISCUSSION.

The rapid method for determining electrical conductivities just described was used in a series of experiments both under field and pot-culture conditions with special reference to the effect of crop growth, of manure, of climatic changes, etc. Altogether some twenty-three arable plots and two grass plots were marked out from which composite samples were collected periodically for measurements of conductivities over a period of 15–20 months. In the pot experiments which were conducted for 2 years with barley, the composite sample was obtained by mixing together soil, taken by means of a cylindrical cork borer $4\frac{1}{2} \times \frac{3}{4}$ in., from four replicate pots. The results of all the field and pot experiments are not given here, but attention is confined to typical ones.

Effect of manure and crop growth.

In Fig. 1 the results of a few pot experiments are given. It was observed that the 7 days' increase of a soil treated with inorganic

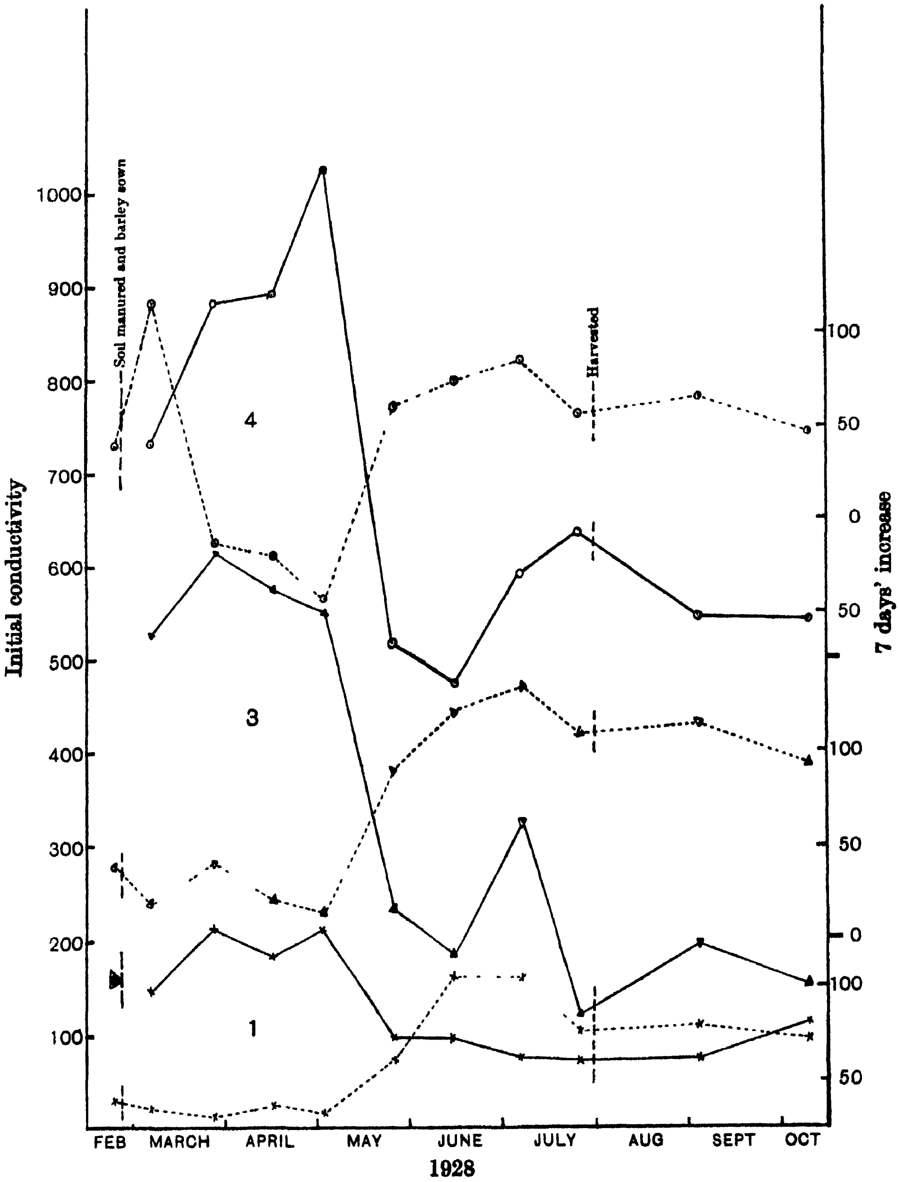

Fig. 1. Showing the changes in the initial conductivity and 7 days' increase of soil in pot experiments:

No.	Manures given to same soil	Initial conductivity	Seven days' increase
1	Nil	×——×——×	×- - - -×- - - -×
3	*I* (= inorganic fertilizers) + *D* (= dung)	▲——▲——▲	▲- - - -▲- - - -▲
4	*I* + *D* + *R* (= Rape cake)	◉——◉——◉	◉- - - -◉- - - -◉

Note. For correct values of the initial conductivity of No. 3 add 250 to values shown in the curve.

fertilisers alone or with dung was slightly smaller than that of the same soil untreated. But when rape cake was applied in addition to other manures a large 7 days' increase was obtained for the first sample taken after the treatment. This increased value for the soil was only temporary, for the value was abnormally low, in fact negative, for the few samples taken subsequently. Since in these pot experiments the first set of samples was not collected until 8 days after the manurial treatment, new pot experiments were tried in which measurements were made on samples taken at short intervals, viz. 3 hours, 1, 3, 5, 8 and 15 days after treating the soil with different manures. No marked rise in the 7 days' increase, but rather a tendency for it to become slightly less than that of the untreated soil was obtained where manures other than rape cake were applied. But where the latter was given, the largest 7 days' increase was obtained for samples collected soon after the treatment, viz. 3 hours and 1 day. For samples taken subsequently the 7 days' increase was progressively smaller, becoming equal to that of the unmanured soil in the course of 15 days, Table III. Bacterial counts in some of the samples are also given in the table.

Table III.

	Un-manured soil	Soil treated with manures including rape cake. Samples taken after					
		3 hr.	1 day	3 days	5 days	8 days	15 days
7 days' increase	42	236	238	223	135	102	36
Bacteria in millions per gm. of soil	6·3	—	160 (0·6)	246 (1·8)	346 (12·8)	374 (18·8)	13 (0·0)

The figures in brackets give the numbers of actinomyces in millions per gm. of soil. The above counts were made on Thornton's agar (36).

Next, to a sample of soil taken after 15 days in the preceding experiment, a little rape cake was added, when a very large 7 days' increase was obtained again. It is obvious, therefore, that the progressive fall in the 7 days' increase of successive samples, as seen in the above table, is due to rapid disappearance of rape cake from the soil. This disappearance was, of course, caused by the micro-organisms, for taking the bacterial counts it will be evident that the added rape cake must have served as sources of energy and food to the bacteria, which therefore multiplied quickly, but in doing so they rapidly used up the rape cake. At the end of the eighth day very little of the added rape cake was left undecomposed. Consequently for want of adequate supply of available energy the majority of the bacteria died during the next few days.

It is clear, therefore, that the presence of decomposable organic matter in the soil tends to increase its 7 days' value. With the growth of the crop the soil becomes richer in decomposable organic matter in the form of dead roots, etc., and it is evident from Fig. 1 that in all cases the 7 days' increase of the soil increased steadily from the middle of the growing period until the crop was fully mature before harvest. Thereafter the 7 days' increase remained more or less constant with a slight tendency to fall.

Taking the initial conductivity of the soil in the pot experiments it will be found that this measurement, as is to be expected, became very high after the application of manure. But in the case of soil under treatment 4 it continued to increase steadily during the next 8–9 weeks in spite of the facts that a crop was growing on the soil and that water was frequently added to keep the soil properly moist. Obviously the removal of soluble salts by the plant during seedling stage was not very marked, nor was there considerable leaching out of soluble salts beyond the sampling depth. Nevertheless the progressive increase in the initial conductivity of the soil was contrary to expectation. An estimation of nitrate was therefore made in the first five samples (all fresh samples were dried in air and preserved carefully in stoppered bottles) which were found to contain 9, 10, 108, 102 and 10 parts of nitric nitrogen per million of dry soil. The explanation of the increase in the conductivity is now seen. After the death of the majority of bacteria for want of energy material when the added rape became exhausted in the soil an increased liberation of plant food took place (10). Another point of interest shown by the above nitrate figures is the considerable drop in the nitrate content of the soil in the fifth sample. On referring to Fig. 1 it will be seen that the initial conductivity of the soil decreased remarkably during the period intervening between the collection of the fourth and the fifth samples, after which it tended to fluctuate round about a mean value. Similar marked fall in the conductivity during this period was obtained in all pot experiments without exception. The result, therefore, is in agreement with those of previous workers (35, 6), who found also that by far the largest amount of soluble plant food material was absorbed by the crop during the middle of the growing season.

On the other hand, the initial conductivity of field soils was not affected by the growth of crop, Figs. 2, 3 and 4. Although in some plots the increased conductivity after the addition of manure gradually fell off, the decrease was rather due to the leaching out of soluble salts beyond the sampling depth than as the result of crop growth. For taking Fig. 3

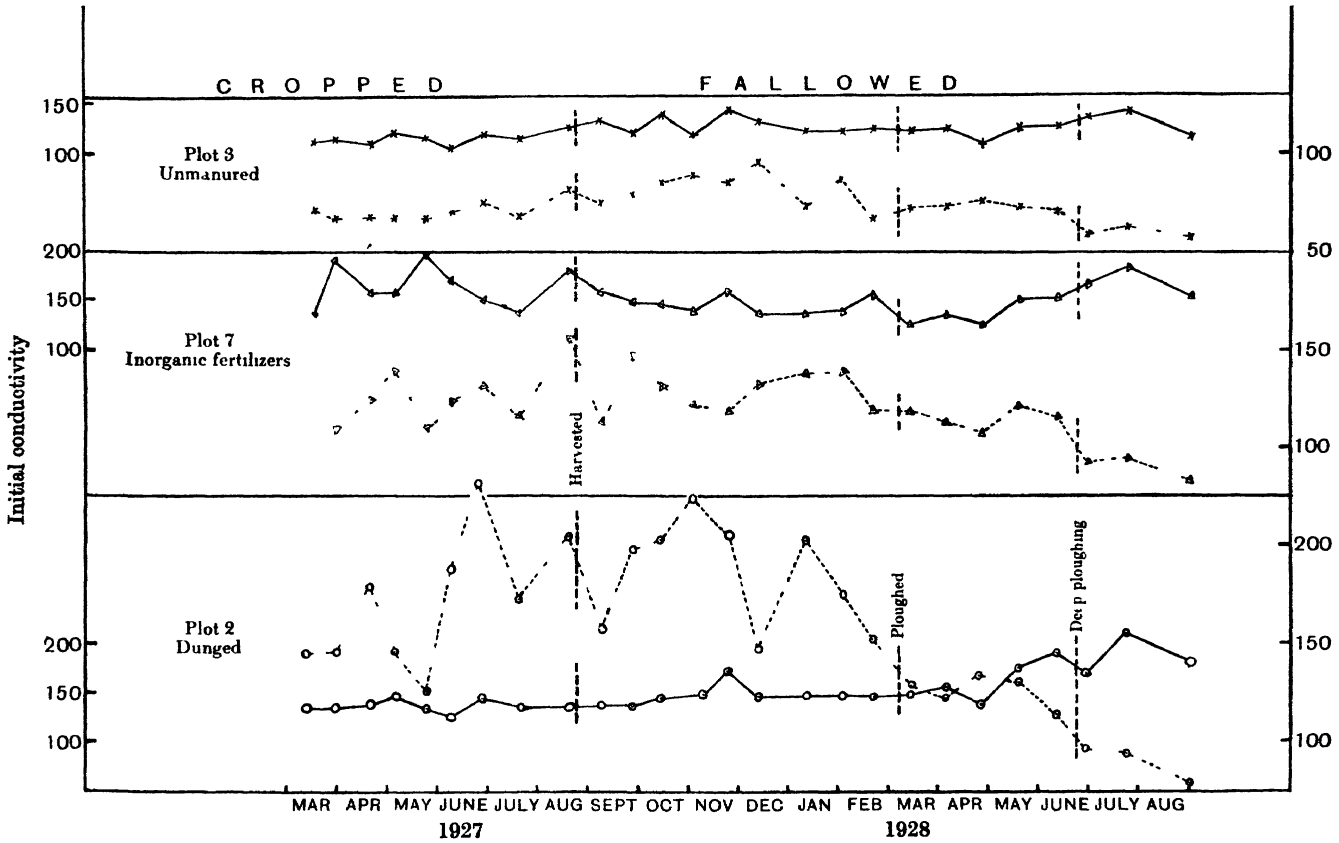

Fig. 2. Showing seasonal changes in the initial conductivity and 7 days' increase of Broadbalk plots. Initial conductivity is represented by straight line and 7 days' increase by broken line. Manure given and wheat sown in early October 1926.

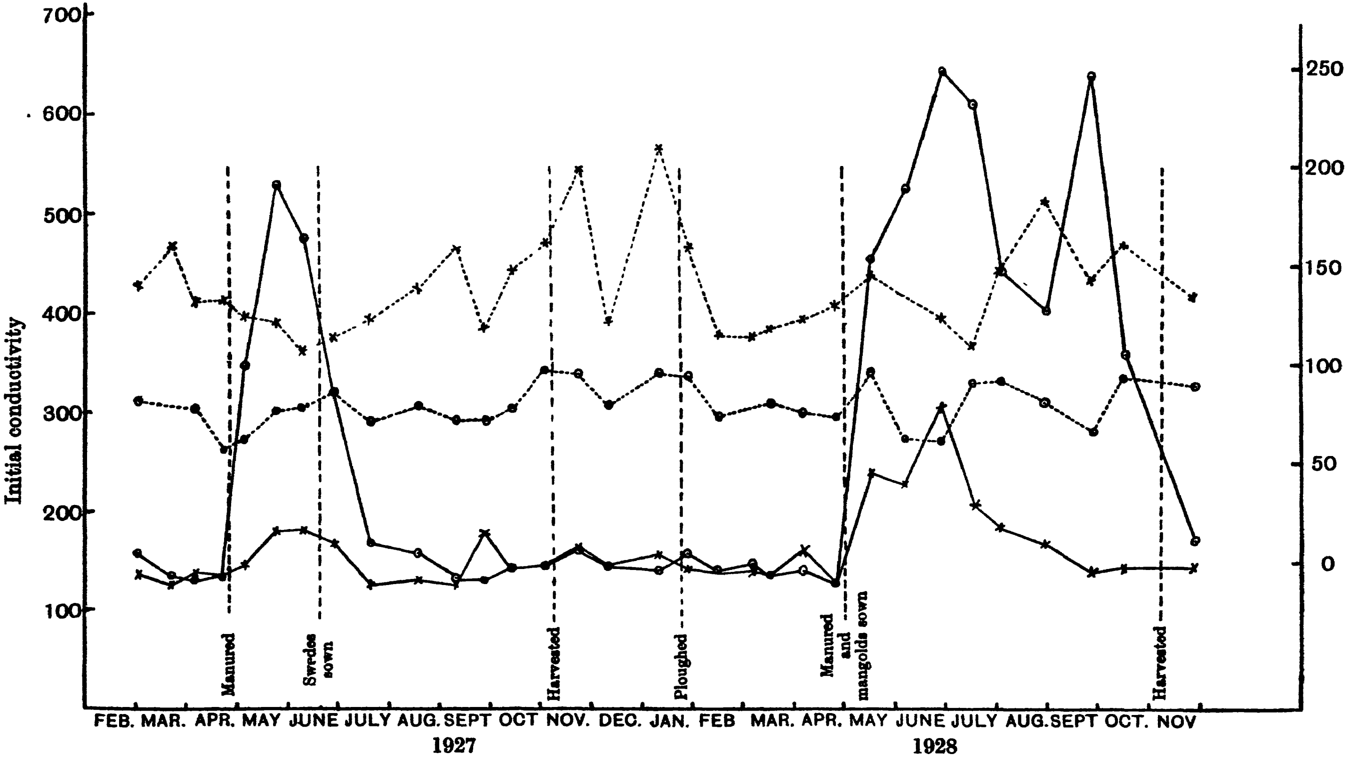

Fig. 3. Showing the seasonal changes in the initial conductivity and 7 days' increase of Barnfield plots:

Plot	Manures given every year since 1876	Initial conductivity	Seven days' increase
1–0	Dung	×——×——×	×----×----×
4–*A*	Inorganic fertilizers	⊙——⊙——⊙	⊙----⊙----⊙

it will be seen that swedes were sown towards the latter half of June in 1927, and by the middle of July, when the crop was scarcely bigger than seedlings, the initial conductivity had already come down to the pre-manured value which changed very little afterwards. The rainfall during the period was moderately high, and it is reasonable to assume that the decrease in conductivity was rather a consequence of this than due to the growth of seedlings. Furthermore, the summer of 1928 was much drier than that of 1927, and the curves show that a much longer period elapsed before the increased conductivity came down to the normal value during the second season.

The apparent contradiction between the results of field and pot experiments regarding the observed effect of crop growth on the initial conductivity is doubtless due to the far heavier growth of crop per unit area in the latter case as will be evident from the following figures for crop yields worked out as bushels per acre.

Table IV.

	Soil manured with			
	Nil	Minerals	Dung	Minerals, dung and rape cake
Broadbalk plots yield in 1927	7	22	24	—
Pot experiments yield in 1928	136	438	—	521

Thus it appears probable that only under heavy cropping conditions does the growth of the plant affect appreciably the easily soluble salt content of the soil.

As regards the changes in the 7 days' increase of soils under field conditions, where the growth of crop was heavy, *e.g.* the dunged plots, the value tended to increase under crop although considerable fluctuations were observed. These fluctuations were to be expected, since the periodic samples which were taken successively at 1 ft. apart were necessarily collected at different distances from the plants, thus giving an irregular amount of decomposable organic matter in the samples. In the case of unmanured plots giving poor yields the fluctuations in the 7 days' increase of the soil were not sufficiently marked at any time throughout the year, for obvious reasons.

Effect of ploughing.

It will be seen from the figures that the 7 days' increase continued to fluctuate for a long time after harvest if the plots were left otherwise undisturbed. But after the plots were ploughed not only did the fluctuations disappear but also the 7 days' increase became equal to the value

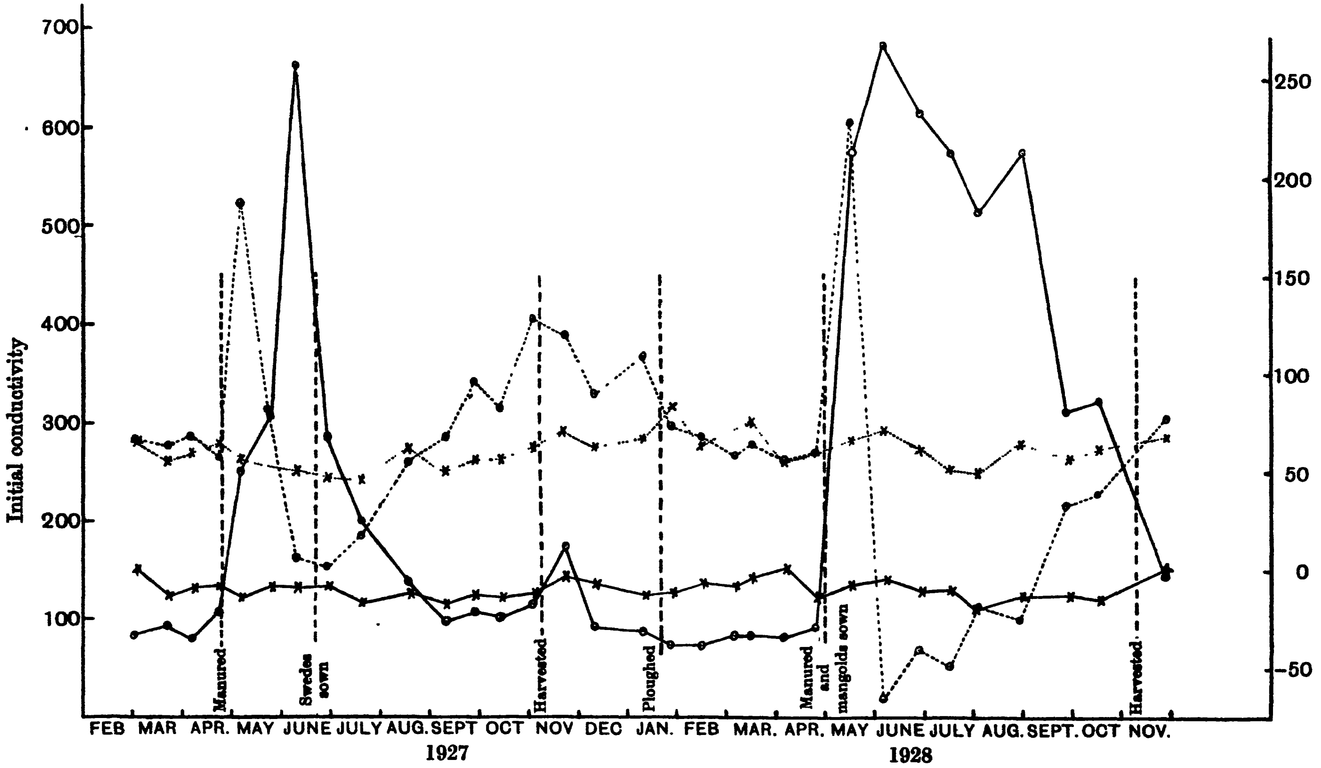

Fig. 4. Showing the seasonal changes in the initial conductivity and 7 days' increase of Barnfield plots.

Plot	Manures given every year since 1876	Initial conductivity	Seven days' increase
8–0	Nil	×——×——×	×----×----×
2–*AC*	Inorganic fertilizers. Dung and rape cake	⊙——⊙——⊙	⊙----⊙----⊙

previous to the lifting of the crop. It appears, therefore, that in the cases of plots bearing annual crop the best time for taking samples for determination of 7 days' increase, if this measurement is to serve as an index of fertility, is when the soil has been prepared for the crop but before manure is applied.

To study the effect of ploughing in more detail the following arbitrary experiment was carried out. A plot 12 × 3 ft. in a permanent grass field was stripped of the top ½ in. of turf, and divided into four plots, 3 × 3 ft., treated as follows:

A and C: dug to 4 in. depth with a spade, clods being broken.

B: dug to 8 in. depth, the 4–8 in. layer being placed over the 0–4 in. layer.

D: undisturbed.

Treatment A and C thus simulated a 4 in. cultivation with a cultivator, while B was analogous to an 8 in. ploughing with good inversion of the furrow.

The fresh composite soil samples were obtained from each plot from a mixture of three holes at 1 ft. apart. The mean of the measurements made on four such samples taken at intervals of 1 week are given below.

Table V.

Plot ...	A	C	D (0–4 in.)	B	D (4–8 in.)
Initial conductivity	167	161	167	139	134
7 days' increase	225	221	240	141	133

It is evident from the above that the measurements for plots A and C were almost equal to those for the 0–4 in. sample of the undisturbed plot D, although the former were dug to a depth of 4 in. and the latter was not. Consequently, ploughing of soils to a depth of 4 in. does not affect the measurements appreciably. On the other hand, the measurements for plot B in which the 4–8 in. soil was brought to the surface were equal to those for 4–8 in. sample of plot D. Consequently for a surface sample taken after deep ploughing the observed change in the measurements will depend on the values for the subsoil. As a rule the subsoil has a smaller 7 days' increase.

Effect of fallowing.

A different portion of each of the three Broadbalk plots reported in Fig. 2 was fallowed continuously for over 2 years. In Fig. 5 measurements on samples taken from these plots are given. It will be seen that continued fallowing had no marked effect on either of the two measure-

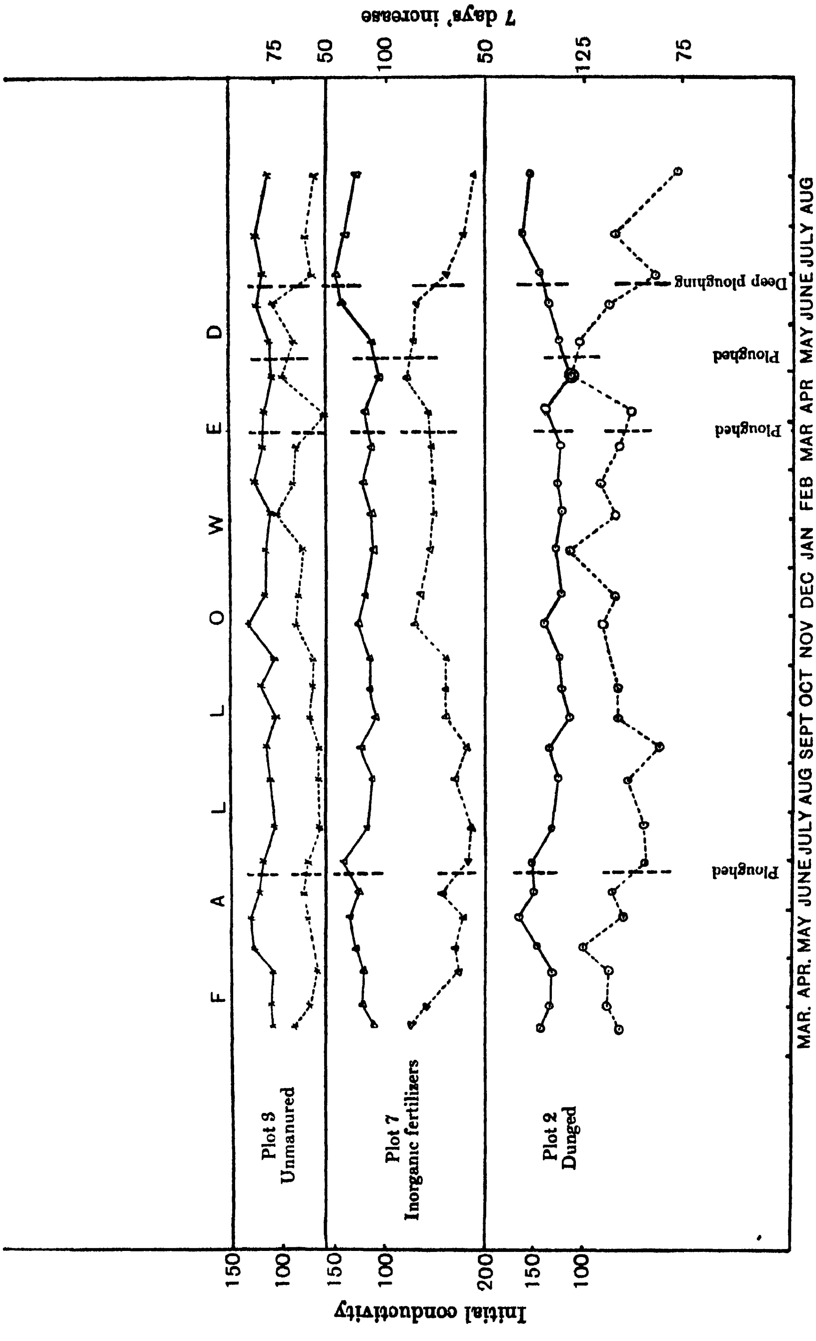

Fig. 5. Showing seasonal changes in the initial conductivity and 7 days' increase of Broadbalk fallow-fallow plots. These plots were manured annually as stated below until 1925 and have been fallowed over the period shown. Initial conductivity is represented by straight line and 7 days' increase by broken line.

ments. The result is probably typical of soils under temperate humid conditions. In the tropics, however, the exposure to the baking heat of the sun is known to bring about considerable changes in the microbiological and physical conditions of the soil (28, 24).

Influence of seasons.

An examination of the figures giving results of arable plots indicated that the influence, if any, of climatic changes on the measurements for these soils had not been very marked. It may be that the seasons had their effects, but the indications of the latter were marred by those of other effects such as of manurial treatments, crop growth, cultivation operations, etc. A permanent grass plot was therefore selected, the soil of which, except for the collection of samples, was not disturbed in any way. A number of composite samples were examined during each season at regular intervals. It was found that although there were differences in the measurements made during one season, the variations, except in the case of spring samples, were not statistically significant, that is to say they were not greater than those accountable by soil heterogeneity[1]. When, however, the seasonal averages of the measurements were considered, the 7 days' increase of the soil was found to increase progressively from winter to summer, but to come down considerably during the following autumn. Both the increase in summer and the drop in the autumn were significant. Since the 7 days' increase becomes larger when a moist soil is air dried the rise and fall in the value were probably due to progressive desiccation of the soil from winter to summer followed by a gain in the moisture content of the soil in autumn, as will be evident from the seasonal average figures given in Table VI.

In addition to the moisture change, the temperature also affects the 7 days' increase, especially of the surface soil. Thus the 7 days' increase of 0–4 in. samples collected when the plot lay frozen for some time in winter was found to be 326, which, as will be seen from Table VI, is much larger than the mean 7 days' value of other non-frozen fresh samples taken during that season. This value of the frozen sample was almost equal to the mean value of the air-dried winter samples, although the moisture content of the former was 41·2 per cent. The result is not surprising, since it is known that slight freezing of the soil helps bacterial development (8, 23, 37) and modifies the colloidal condition of the soil, and it may thus have the same stimulating action as air drying (38). When the mean seasonal temperatures[1] and the mean seasonal 7 days' increase of 0–4 in. and 4–8 in. soils are plotted against each other the graph was found to be almost a straight line, indicating thereby that in general an increase of the temperature in the soil *in situ* tends to affect the 7 days' increase favourably.

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[1] The standard error due to soil heterogeneity was calculated (11) from measurement for three separate composite samples collected on a particular day in winter and also in autumn.

Table VI.

	Fresh soil samples				Air-dry samples
	0–4 in.		4–8 in.		0–4 in.
	7 days' increase	% of moisture	7 days' increase	% of moisture	7 days' increase
Winter	191	39·5	144	29·5	324
Spring	271	24·8	141	20·9	331
Summer	407	13·3	284	10·6	384
Autumn	266	23·5	196	16·5	311

It has already been stated that though in general the variations in the measurements within any one season were not significant the spring samples showed some exceptions to this general observation. This may be attributed to the progressively warmer weather and scanty rainfall which prevailed during that period. This resulted in steady decrease in the soil moisture and an increase in the average soil temperature, both of which went to affect the measurement. Consequently within any short period of time the changes in the amount of sunshine and of rainfall may bring about significant variations in the 7 days' increase provided that they are sufficient to modify the soil temperature or moisture appreciably. Since in general various meteorological factors vary simultaneously it was thought that no useful purpose would be served by attempting to estimate the effect of any one of them in individual cases.

The "negative" 7 days' increase.

It has been stated above (p. 219) that a few weeks after the soil was treated with rape cake, the 7 days' increase of the soil became negative, that is to say on allowing to stand, the conductivity of the suspension actually decreased during the next 7 days. This unexpected result which was obtained in the case of both pot and field experiments (Figs. 1 and 4) where rape cake was used requires explanation. Further experiments were conducted, therefore, with soil suspensions to which instead of rape cake a simple but easily decomposing substance, viz. glucose, was added in different doses. These suspensions were prepared from portions of the same soil which had been stored in moist conditions for 12 weeks in stoppered bottles after being treated with different manures as shown in Table VII. The nitrate contents of the soils at the end of 12 weeks' storage are also given in Table VII.

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[1] Averages of daily temperatures taken during the period of observation in each season, the daily temperature being a mean of three temperatures recorded at 9 a.m., 3 p.m. and 9 p.m.

Table VII. *Showing the changes in the 7 days' increase of soil suspensions containing different amounts of a 5 per cent. glucose solution.*

Soil sample no.	Treatment	Suspension with no glucose: Initial condition	Suspension with no glucose: 7 days' increase	7 days' increase of the suspension when the amount of glucose solution added was: 1 c.c.	3 c.c.	7 c.c.	Nitrate parts per million
1	$A = K_2SO_4$ + superphosphate	377	35	184	516	904	12
2	$B = A + (NH_4)_2SO_4$	584	57	66	353	—	58
3	$C = B$ + dung	777	28	40	262	909	67
4	$D = C$ + cake	993	34	-109	146	657	75

N.B. The initial conductivities of the soil suspensions remained practically unchanged after the addition of glucose solution.

It is evident from the above table that the 7 days' increase of a suspension becomes greater as the amount of glucose added to it is increased. But the most striking fact is that with the lower doses of glucose (1 c.c. and 3 c.c.) the magnitudes of the 7 days' increase of the suspensions were in the reverse order to their initial conductivities and the nitrate contents of the soils. An explanation for this is possible only from the biological point of view. For any given quantity of energy material added to the soil suspension, a certain amount of nutrient is assimilated by the organisms in building up their cell substances. The larger the fraction of the total nutrient assimilated which is present in the suspension in the soluble form at the beginning the greater will be the quantity of such substances rendered insoluble during the 7 days' standing after the addition of glucose, and therefore the smaller will be the 7 days' increase. It is highly probable, in view of the fertiliser treatment shown in Table VII, that the suspensions of the stored soils showing the higher initial conductivities contained larger amounts of nutrients in solution, and the assumption is strengthened by the figures for the nitrate content of the soils, the principal substance assimilated as nutrient.

Proceeding a stage further, three factors bearing on the electrical conductivity after the addition of glucose solution may be considered:

(1) Glucose itself does not affect the conductivity, but its decom-

position products do. Consequently the 7 days' increase will be favoured by its decomposition.

(2) As stated above the presence of glucose will help assimilation of some of the electrolytes already in solution, and consequently this part of micro-organic activity is distinctly unfavourable to the 7 days' increase.

(3) After the dissolved inorganic nutrients have been completely utilised the presence of any remaining energy material will cause the micro-organisms to attack and decompose some of the insoluble substances present in the soil. As some of the substances thus liberated are soluble such activities will favour the 7 days' increase. This result is obviously greater the more glucose solution is added.

Thus it is seen that on the addition of glucose or other easily decomposable organic substance to soil suspension, the actual 7 days' increase is the balance between the changes in conductivity due to the effects in (1) and (3) and those due to the effects in (2). If a soil is rich in nutrient ionic content such as nitrates, the demand of nutrient arising out of a small dose of glucose may be met completely from that already in solution, thus eliminating the third factor. In such a case the effect of the second factor may overbalance that of the first, and a negative 7 days' increase will result. With higher doses of glucose in the same suspension not only will the effect of the first factor be increased but also the third factor will be brought into play, so that in this case the effect of the second factor may be compensated by the combined effects of the first and the third leading to a positive 7 days' increase. Thus the addition of any quantity of glucose over and above that required to produce a balance between these three effects will always result in a positive 7 days' increase, and the greater the quantity, the greater will be the increase. This was shown clearly in Table VII by the 7 days' increases of soil No. 4 on the addition of 3 c.c. and 7 c.c. of glucose solution.

These processes of assimilation, decomposition, etc., affecting the conductivities of the suspensions on the addition of glucose, are reflected more clearly in the measurements at short intervals which were made at 1, 3 and 5 days' standing in the experiment reported in Table VII as shown in Table VIII.

Taking soil No. 4, which contained the largest amount of nitrate and other soluble salts (Table VII), it is seen from Table VIII that the amount of electrolytes assimilated by micro-organisms in the presence of added glucose during the first day was not compensated by the amount released as a result of various decompositions; but whereas the balance remained unfavourable throughout when the quantity of glucose added was 1 c.c., in spite of a distinct tendency to recovery at the end of the third day, it was reversed in the presence of larger doses, and the larger the dose the more quickly the balance became favourable.

Table VIII.

Suspension of soil no.	Amount of 5 % glucose solution added c.c.	Total increase in conductivity on allowing the suspension to stand for 1 day	3 days	5 days	7 days
4	1	-51	-129	-107	-109
4	3	-55	- 10	112	146
4	7	43	235	490	657
3	3	-12	169	265	262
3	7	-15	489	766	909
1	3	33	329	511	516
1	7	21	416	790	904

With soil No. 3, which contained less nitrate and less amount of other soluble salts, though the balance was slightly unfavourable at the end of 1 day with both doses of the glucose solution it was much less so than in the case of soil No. 4. Later on the increases were positive.

With soil No. 1, which contained the least amount of soluble salts and very little nitrate, the balance was never unfavourable with either of the two doses of the glucose solution.

CONCLUSION.

It has been shown that as a result of microbiological activities in the presence of energy materials, release of electrolytes and removal of some ions take place simultaneously in soil suspensions. In normal soils a certain amount of energy material is always available, and consequently the actual change in the electrical conductivity of a soil suspension in a given period of standing (say 7 days) is the balance between the increase in conductivity due to release of fresh electrolytes and the decrease due to the assimilation or conversion of electrolytes present initially in the suspension into such forms which do not affect the conductivity. In soil suspensions containing a large quantity of nutrient elements, such as nitrates in solution, the presence of a small quantity of organic matter, rich in energy in an easily available form, may cause an unfavourable balance, *i.e.* the conductivity may actually decrease on allowing to stand for 7 days. However, in normal field soils no excess of easily decomposable organic matter can remain long, and in the course of a few weeks, depending on circumstances, a normal condition will be restored. This is well known from the fact that the carbon-nitrogen ratio in field soils tends always to attain a constant value of about 10 to 11. From this it follows that if the collection of soil samples from a plot be postponed for some time after the ploughing of stubbles or the application of manure (particularly of organic and an easily decomposable type such as rape cake) so as to allow the biological activities in the soil to readjust themselves to normal condition, a positive 7 days' increase of suspensions of soil from that plot should be obtained. This is found generally to be the case. Under such conditions a large 7 days' increase will indicate that the normal condition of that soil is favourable to biological activities and therefore to the rapid liberation of plant food. Such a soil may therefore be expected to have a higher inherent fertility than one in which the 7 days' increase is smaller.

SUMMARY.

Several factors affecting the electrical conductivity of soil suspensions have been studied leading to the development of a simple and rapid technique for its measurement, and suitable for both moist and air-dry soils.

The possible use of the method in soil fertility studies necessitated examination of the effects of cultivations, manures, crop, meteorological variations, etc., on the conductivity measurements. This involved periodical determinations of initial conductivity (*i.e.* the specific conductivity of a 1 to 5 aqueous soil suspension determined at 25° C.) and the 7 days' increase (*i.e.* the rise in specific conductivity of the same aqueous suspension after standing for 7 days in a thermostat at 25° C.). Soil samples were examined from several Rothamsted classical and non-classical plots (over twenty plots were investigated for 15–20 months), and samples from experiments were also utilised during two growing seasons. As considerable changes in soil conditions were found to take place on air drying, all the above measurements were made on fresh soil samples. The results showed that:

(1) There is practically no seasonal change in the measurements for unmanured plots giving poor yield.

(2) No appreciable reduction takes place in the initial conductivity (*i.e.* soluble salt content) of the soil by the growing crop in manured plots unless the growth is very heavy. In the latter case considerable reduction takes place during only the middle period of the growth. Most of the excess salt in the soil after treatment with manure is washed down in the course of a few months, depending on rainfall, beyond the sampling depth.

(3) The 7 days' increase of soil tends to decrease slightly following the application of inorganic fertilisers, but is profoundly affected by the addition of easily decomposing organic manure such as rape cake or by the presence of dead roots and stubbles in the soil. In general these substances increase the 7 days' value, but if the soil is rich in nutrient ionic content such as nitrate the presence of energy materials may cause a negative 7 days' increase.

(4) Continued fallowing of soil has little or no effect on the measurements.

(5) Ploughing, especially when deep, affects the measurements by bringing subsoil to the surface.

(6) In experiments on arable soils where the measurement of 7 days' increase is to serve as an index of fertility, samples should not be taken until some time after the application of manure, nor while the plot is under crop. The best time is when the soil has been prepared for the crop but before manure is applied.

(7) For soil under permanent grass there are marked seasonal variations in the 7 days' increase of surface soil. For comparisons in this case therefore, soil samples should be taken at the same season of the year and preferably under similar meteorological conditions.

A large 7 days' increase of a soil collected as stated in (6) and (7) above will indicate that the normal condition of that soil is favourable to biological activities and therefore to the rapid liberation of plant food. Such a soil may therefore be expected to have a higher inherent fertility than one which has a smaller 7 days' increase.

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MEASUREMENTS OF THE ELECTRICAL CAPACITY AND CONDUCTIVITY OF SOIL BLOCKS.

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(With Six Text-figures.)

Introduction.

On account of the influence of moisture content on the physical properties of soil, many laboratory studies have been made on the variation with moisture of those of importance in cultural operations, or in such problems as the movement and distribution of water in soils. Matters are complicated by the fact that soil properties depend not only on the moisture content but also on previous moisture changes, as any given moisture may be approached from a higher or lower value: for example, volume changes of soil are not reversible on drying and rewetting if the soil has been dried out below a certain limit. In laboratory work it is most convenient to obtain a series of moisture contents by starting with a wet soil and allowing it to dry out to varying extents, but even under these conditions the results of different investigations often disagree, some suggesting that the variation with moisture content is continuous, others that the variation is essentially a discontinuous process.

Atterberg (1) examined the behaviour of soils over wide ranges of moisture content and divided the moisture range for each soil into six main parts. In any particular moisture region the soil exhibits a definite "consistency" or behaviour towards outside forces such as gravity and pressure, and each consistency is distinguished from the others by the different effects on the soil of these forces, or by the "feel" of the soil. Atterberg's work implies that changes in the properties of soils occur at well-defined moistures, and in recent years some of these have been used by soil workers as "single value" constants, in an attempt to classify the general characteristics of a soil by a single number.

From the point of view of the work to be described, the three most important of Atterberg's limits are the sticky point (die Klebegrenze), the lower plastic limit (die Ausrollgrenze), and the moisture content at which air enters the soil pores (die Schwindungsgrenze). The sticky point is determined by first mixing the soil with water until it is definitely wet and will stick to a nickel spatula. The soil is then kneaded by hand until the point is reached when it no longer adheres to the spatula when the latter is drawn across or through it. The lower plastic limit is defined as the moisture content below which the soil can no longer be rolled into a fine wire under the finger without crumbling. Below this limit the soil crumbs will adhere when pressed lightly together, and will shrink on drying without entry of air into the soil pores. When, however, enough water has evaporated the film breaks between the soil particles, the colour changes, and shrinkage ceases. Atterberg (2) also obtained a break in the cohesion-moisture curve at this limit. For lower moistures the soil crumbs will not adhere when pressed lightly together, but if the soil has been moulded when wet and allowed to dry out below this limit, then the force required to crush or break the soil may be considerable.

Haines (3, 4) confirmed the slowing down in the rate of shrinkage, but could find no discontinuity in his cohesion measurements, although he used the same type of apparatus and essentially the same method. The cohesion measurements entail the preparation of a series of test blocks, and as it is impossible to obtain them identically alike the errors in this type of experiment are large: for this reason it is difficult to detect a small change in the variation of cohesion with moisture on the entry of air into the soil, should such a change occur. In order to obtain more accurate data on soil moisture relationships, some type of experiment in which one sample of soil can be used throughout was obviously required, and for this purpose an electrical method is most convenient. Measurements can be made comparatively accurately, and it is reasonable to suppose that any changes, depending on moisture, in the mechanical properties of soils will be reflected in some way in their electrical properties.

PREVIOUS CONDUCTIVITY MEASUREMENTS.

Conductivity determinations have been used chiefly in attempts to develop methods of measuring either moisture or salt contents under field conditions. Laboratory measurements on the change of conductivity with moisture content have been made by Whitney and Means (5), Deighton (6) and Haines (7).

Whitney and Means measured the specific resistance of their soils in a hard rubber cell with brass electrodes, the soils being mixed with water and packed into the cell for each determination. Deighton's method was similar except that for electrodes he used carbon, which is self-depolarising to some extent owing to its ability to absorb gases. As Haines pointed out, these workers were probably altering the degree of packing and the contact with the electrodes for each measurement as well as the moisture content. Consequently, Haines measured the change in resistance taking place, as a block of soil, moulded at the sticky point, was allowed to dry out, and used mercury electrodes to improve the soil-electrode contact. There is one criticism which may be advanced against this technique and that is the possibility of ageing effects.

The results of Whitney and Means gave straight lines or very slightly bent smooth curves for a moisture range of 6 to 18 per cent., the resistance varying inversely as the square of the moisture content. Deighton confirmed the latter law for moistures above 10 per cent., but below this value his results gave one and possibly two discontinuities. Deighton suggested that these might be due to some process analogous to the coagulation of soaps, as Laing and McBain had found that the specific conductivity of soap curds was lower than that of either the sol or gel.

Haines found that for natural soils the conductivity curves were smooth and more or less concave to the moisture axis, with no indication as to when air entered the soil. Some heavy clays and a sample of kaolin, however, formed an exception. The conductivity remained constant for a considerable range of moisture content, and only began to fall when entry of air into the soil took place. The constancy of conductivity when the pore space was filled with water seems remarkable if the conductivity is regarded simply as the usual electrolytic conductivity of a solution: for it might be expected that the shrinkage of the soil would give rise to a mechanical obstruction to the movement of the ions and so cause a decrease in conductivity. It is known, however, that the ions present in the soil solution are attracted towards the soil water interface, their concentration decreasing with distance from the interface. On the application of an alternating field, the ions move over the surface of the soil particle, and since the force of attraction to the interface varies for different ions, their response will depend on the restoring force, the applied field and its frequency. The net effect of their movement will be similar to the passage of an alternating current through a condenser, and will give rise to a difference in phase between the applied E.M.F. and the current flowing. When measuring the conductivity of such a system by a bridge method it is necessary to introduce a compensating capacity either in series or in parallel with the variable arm of the bridge. In the experiments described below the values of the parallel capacity required for balance were measured.

EXPERIMENTAL.

The technique of preparation of the soil blocks was the same as that employed by Haines. After passing the soil through a sieve with 100 meshes to the inch, distilled water was added until the moisture content was approximately that of the sticky point. After thorough mixing, rectangular blocks of soil were made by means of brass moulds and the electrodes inserted. For purposes of comparison both carbon and mercury electrodes were used. Ideally the electrodes should be of the same cross-section as the block, but, owing to shrinkage, it is not possible to maintain a constant contact, and the carbon electrodes employed were cylindrical rods of approximately 1·5 mm. diameter. The system is complex in the sense that besides the resistance and capacity of the soil there are resistance and capacity associated with the electrode-soil interface. The measurements taken, *i.e.* the parallel capacity and resistance, give the equivalent electrical circuit of the soil and interface effects combined. An attempt was made to separate them by using three electrodes at different distances apart, for, assuming that each electrode introduces the same impedance, it should be possible to calculate the phase angle of the current in the soil.

In a preliminary series of measurements the soils were allowed to dry out slowly, the experiments lasting approximately 4 weeks so that the moisture distribution in the block should remain as uniform as possible. In his paper on the shrinkage of soils Haines has shown that under such conditions the difference in moisture for the inside and the outside layers is small.

The apparatus consisted of a valve oscillator, a Cambridge Scientific Instrument Company bridge, and a variable condenser, earphones being used as detector. One corner of the bridge was earthed. The condenser was made from a series of T.C.C. mica condensers ranging in value from 0·0001 to 1·0 μF and an Ormond 0·001 μF variable condenser. Each fixed condenser could be connected in parallel with the Ormond, and all were calibrated in terms of one of them chosen as standard. With this apparatus sharp minima of sound could be obtained in the earphones, but the method of earthing the bridge was unsatisfactory, and at low moistures the current flowing through the soil block was large enough to produce slight heating effects as the measurements were being made.

Consequently, in the second series of experiments the oscillator was modified to give a smaller output, and a two-stage amplifier was employed to provide the necessary sensitivity. The technique was further improved by earthing the bridge in the manner described by Jones and Josephs(8).

This method ensures that the telephone is kept at earth potential when in balance, and also eliminates errors due to unsymmetrical capacitance from the oscillator or oscillator leads to earth. The final form of the apparatus is shown in Fig. 1. The input balancing device consists of a series of resistances approximately equal to those of the ratio arms of the bridge, and a variable condenser K. Any pair of these resistances could be joined in series with the slide wire CC', and either input lead connected to earth through the condenser K by means of a switch (S_1). In this apparatus K was a Ton-a-cap variable condenser and the resistances were of manganin wire wound non-inductively on ebonite plates.

In using the bridge, one end of the telephones is first connected to earth by a switch (S_2), and the point B of the main bridge brought to earth potential by adjusting the position of the contact on the slide wire and the setting of the condenser K. When a balance has been obtained, S_2 is reversed, connecting the telephones across the main bridge, which is then balanced. The setting of the balancing system is checked, and, if necessary, the readings on the bridge corrected.

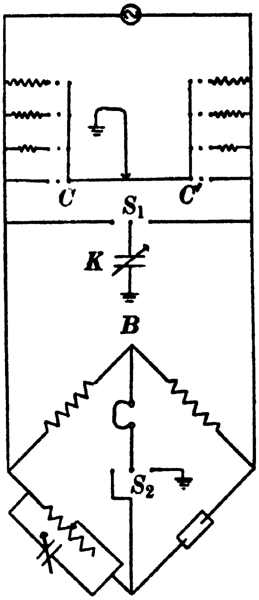


Fig. 1.

In a second series of measurements, the range of the experiment was extended to prove the presence, or absence, of ageing effects by allowing half the number of blocks for each soil to dry out at a faster rate than the remainder.

Experimental results.

Before describing the results obtained for soil, capacity effects observed in the measurement of the resistance of electrolytes will be considered. When, for example, a current is passed between platinum electrodes immersed in dilute sulphuric acid, a back electromotive force is set up and the electrodes are said to be polarised. For alternating current the system can be represented by a resistance R in series with a capacity K, where R is the resistance of the electrolyte and K is the capacity of the polarised electrode. The vector impedance of the system for an alternating current of frequency $\frac{\omega}{2\pi}$ is given by

$$z = R + \frac{1}{j\omega K},$$

and the current leads the E.M.F. by a phase angle $\tan\theta = \frac{1}{\omega RK}$. Thus the effective resistance is R and the reactance $-\frac{1}{\omega K}$.

In bridge measurements the compensating capacity is usually placed in parallel with the variable arm of the bridge, and the vector impedance of a resistance S in parallel with a capacity C is given by

$$z = \left(\frac{1}{S} + \frac{1}{\frac{1}{j\omega C}}\right)^{-1} = \frac{S - j\omega S^2 C}{1 + \omega^2 S^2 C^2},$$

so that for this system the phase angle is given by $\tan\theta = \omega SC$, and the effective resistance and reactance by

$$\frac{S}{1 + \omega^2 S^2 C^2} \quad \text{and} \quad -\frac{\omega S^2 C}{1 + \omega^2 S^2 C^2}$$

respectively.

When the bridge has been balanced the two circuits are equivalent and the corresponding quantities are identical. The effective resistances must be equal, *i.e.*

$$R = \frac{S}{1 + \omega^2 S^2 C^2},$$

or, since the same phase angles are the same,

$$R\left(1 + \frac{1}{\omega^2 R^2 K^2}\right) = S.$$

From the latter equation it is seen that the measured resistance differs from the true resistance R, and that for any given frequency the difference is larger, the smaller the product RK. The correction required may be made negligible by increasing RK to a suitable value, and, when small values of R are being measured, K should be made as large as possible. In practice this condition is realised by platinisation which produces a mass of spongy platinum and greatly increases the effective area of the electrode. For such electrodes the capacity may be of the order of thousands of microfarads per square centimetre, while for bright platinum it is twenty-five to thirty times less. The actual value depends on the concentration of the solution and decreases with increasing frequency. In the case of mercury, the value of the capacity, as calculated from capillary electrometer measurements (*i.e.* for static conditions), is about 30 microfarads per square centimetre, so that for alternating current the value will be somewhat smaller.

Consequently, in the present work with soil, it would be expected that the small series capacity associated with the mercury electrode would require a comparatively large parallel capacity for balance, and that most of the measured parallel capacity would be due to the electrodes. Carbon, being porous, would present a greater surface area, and for this electrode the capacity should be larger and require a smaller balancing capacity. The experimental results show that the parallel capacity measured for the soil blocks with mercury electrodes was always greater than for the soil with carbon electrodes.

For mercury electrodes in the first series of experiments, the general result for falling moisture is an increase in parallel resistance, an initial decrease in parallel capacity followed by a sharp rise to a maximum, after which it falls again, showing two changes in direction at lower moistures. The parallel capacity-moisture curve for a Punjab soil is shown in Fig. 2.

In view of the preceding discussion on electrode effects the parallel capacity has been converted into the equivalent series capacity by means of the equation

$$K = C\left(1 + \frac{1}{\omega^2 S^2 C^2}\right),$$

assuming that the electrode capacity is responsible for the whole of the reactance. The series capacity-moisture curves for the Punjab soil and a Long Newton clay are given in Fig. 2, both curves exhibiting a striking drop in series capacity which is steepest when air begins to enter the soil pores. For the Punjab soil this occurs between moistures of 20 and 18 per cent. The curve also shows that the series capacity decreases over the moisture range of 23 to 20 per cent., while at still higher moistures there is a marked fall, to which reference will be made in a later part of the paper. For high moistures, a calculation from the data for the electrodes at different distances apart shows that the parallel capacity due to the soil is not negligible compared with that due to the electrode, so that the series capacities given are less than those actually existing at the electrode. At the beginning of the experiment, the true series capacities are of the order of 15 microfarads per square centimetre, a value comparable with that found for the capillary electrometer.

Below a moisture of 18 per cent. the series capacity remains comparatively constant, until at 6 per cent. it again decreases rapidly. If plotted on a larger scale, this portion of the curve shows that the capacity is decreasing with decreasing moisture and that a change in its variation with moisture occurs at 13 per cent. This is shown for the second series of

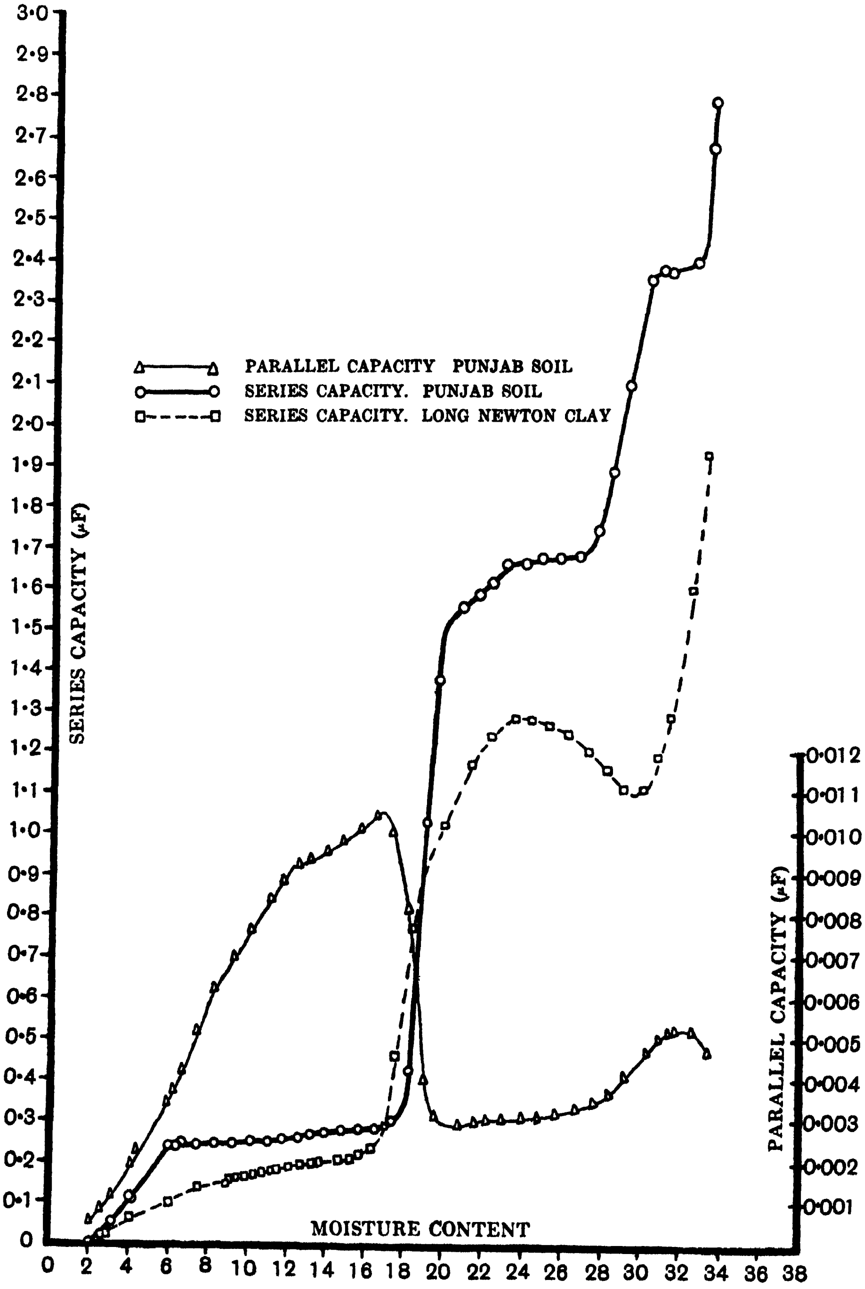

△———△ PARALLEL CAPACITY PUNJAB SOIL
○———○ SERIES CAPACITY. PUNJAB SOIL
□- - - -□ SERIES CAPACITY. LONG NEWTON CLAY
SERIES CAPACITY (μF)
3·0
2·9
2·8
2·7
2·6
2·5
2·4
2·3
2·2
2·1
2·0
1·9
1·8
1·7
1·6
1·5
1·4
1·3
1·2
1·1
1·0
0·9
0·8
0·7
0·6
0·5
0·4
0·3
0·2
0·1
0
PARALLEL CAPACITY (μF)
0·012
0·011
0·010
0·009
0·008
0·007
0·006
0·005
0·004
0·003
0·002
0·001
MOISTURE CONTENT
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
32
34
36
38

Fig. 2.

experiments (when more readings were taken for the lower moistures) in Fig. 5. It will be seen that the final fall in series capacity commences at a moisture between 7 and 8 per cent.

Similarly for the Long Newton Clay the series capacity decreases for moistures below 25 per cent., the rate of change being greatest from 18 to 16 per cent. when entry of air begins. Again, there are two further moistures, 13 and 7 per cent. approximately, where changes in its variation with moisture take place.

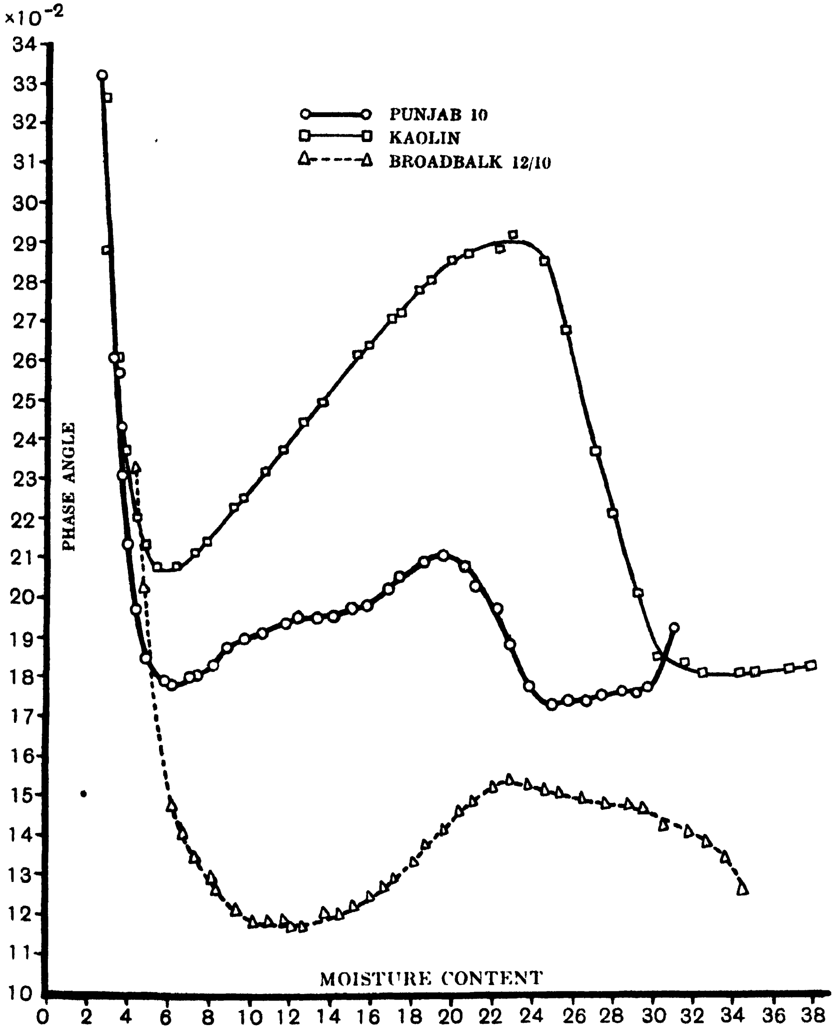


Fig. 3.

For carbon electrodes in the first series of experiments the general result for falling moisture is an increase in parallel capacity. This is true for a series of soils and a sample of kaolin, the only exception being the heavy Long Newton clay, for which the parallel capacity rises to a maximum at an intermediate moisture and afterwards falls rapidly. The soils are perhaps best compared by considering the variation of the phase angle with moisture content, shown in Fig. 3, for two soils and the kaolin. All the curves exhibit a maximum at a moisture content which is approximately that at which air enters the pores, and at lower moistures the phase angle passes through a minimum, after which it rises rapidly. The curve for the Punjab soil shows changes at 12 and 8·5 per cent. corresponding to those observed in the measurements with the mercury electrodes. For this soil and the kaolin the minimum occurs at 6 per cent., while for the heavier Broadbalk soil the phase angle rises for moistures lower than 12 per cent. On the other hand, the phase angle for the still heavier Long Newton clay rises for most of the moisture range, the curve showing discontinuities at moistures of 26, 19, 12 and 7 per cent. approximately.

The second series of experiments with the improved technique gave similar results in all cases where carbon electrodes were used. With mercury electrodes the results are the same except for the Long Newton clay: the parallel capacity required was extremely large and showed a curious reversal in its variation with moisture content. In this series a decrease occurred on the entry of air, whereas in the first there was an increase. Consequently the series capacity rises from a value of 0·3 microfarads per square centimetre to a value of 0·6 microfarads after air had entered the soil pores.

The data obtained shows that the variation of the electrical properties of the soil blocks with decreasing moisture is not continuous, the mercury electrodes being most suitable for detecting these effects, and giving the same general type of result for widely varying soils. The changes observed in the series capacity can only be caused by changes in the contact between the soil and the electrode, and these in turn must depend on the manner in which the moisture is held by the soil: a determination of the lower plastic limit by the rolling technique gives a value of between 22 and 23 per cent. for the Punjab soil, and a fall in series capacity occurs at 23 per cent. Similarly, a shrinkage experiment shows that air begins to enter the soil at 19·5 per cent. moisture, and the main drop in series capacity commences at approximately 20 per cent. This decrease is practically complete in the moisture range 20 to 18 per cent. and, since only a small fraction of the water in the soil has been replaced by air, it can only be explained by the spreading of a film of air between the soil and the mercury. Contact occurs over a small area of the soil particles, and if this area remains approximately constant the small change in series capacity for a considerable moisture range below 18 per cent. is explained. Similarly, such a decrease in contact area should cause an increase in the resistance between the soil and the electrode: a marked fall in conductivity does occur on the entry of air, as shown in Fig. 5, for the slow rate of drying. The series capacity finally falls as the moisture is removed from the surface of the soil particles. The slight change at 12 per cent. is probably due to a series of larger capillaries having filled with air, this moisture marking the extension of the process to the finer ones in the soil. This suggestion is borne out by a more rapid change in the slope of the conductivity curve at this point.

Similar reasoning applies to the Long Newton Clay, where the results for the series capacity are confirmed by the phase-angle curves for the blocks with carbon electrodes. The lower plastic limit occurs at a moisture of 26 per cent. and entry of air at 18 per cent. At present only a tentative explanation can be advanced for the anomalous behaviour in the second experiment when the series capacity increased. The explanation for the decrease generally observed is based on a decreased area of contact between the soil and the electrode. On the same hypothesis, an increase in series capacity could only be caused by an effective increase in the area of contact. This might arise if the surface of the soil were originally contaminated by a film of conducting grease which was drawn into the soil pores. The conductivity did not show the sudden decrease observed in the first experiment, which is in keeping with the above suggestion that the effective contact area was not, at any rate, decreased.

With carbon electrodes the results obtained vary more with the type of soil than is the case with mercury. Since carbon can absorb water the demarcation between the soil and the electrode is less distinct than with mercury, and possibly exceptional moisture conditions may arise near the electrodes. These would depend on the texture of the soil, and may account for the difference in the phase-angle curves for the Punjab soil and the Long Newton clay. As recorded in the experimental section, an attempt was made to separate the soil and electrode effects by using three electrodes in each soil block at different distances apart. The phase angles were always greater for the electrodes closer together, showing that the electrodes do introduce some impedance. When the phase angle of the current in the Punjab soil was calculated from the two sets of readings, the variation with moisture was similar to that for the phase angles

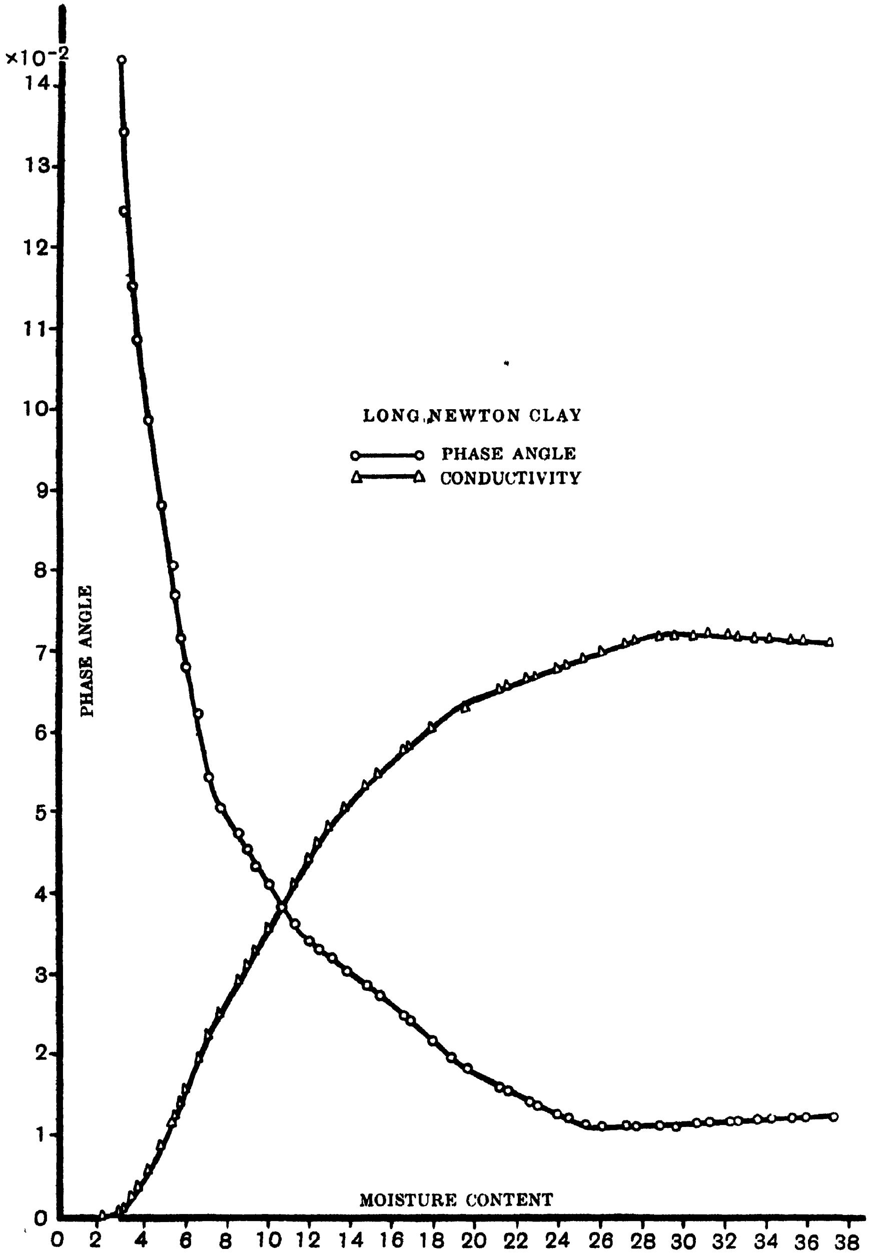

×10⁻²
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
PHASE ANGLE
LONG NEWTON CLAY
PHASE ANGLE
CONDUCTIVITY
MOISTURE CONTENT
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38

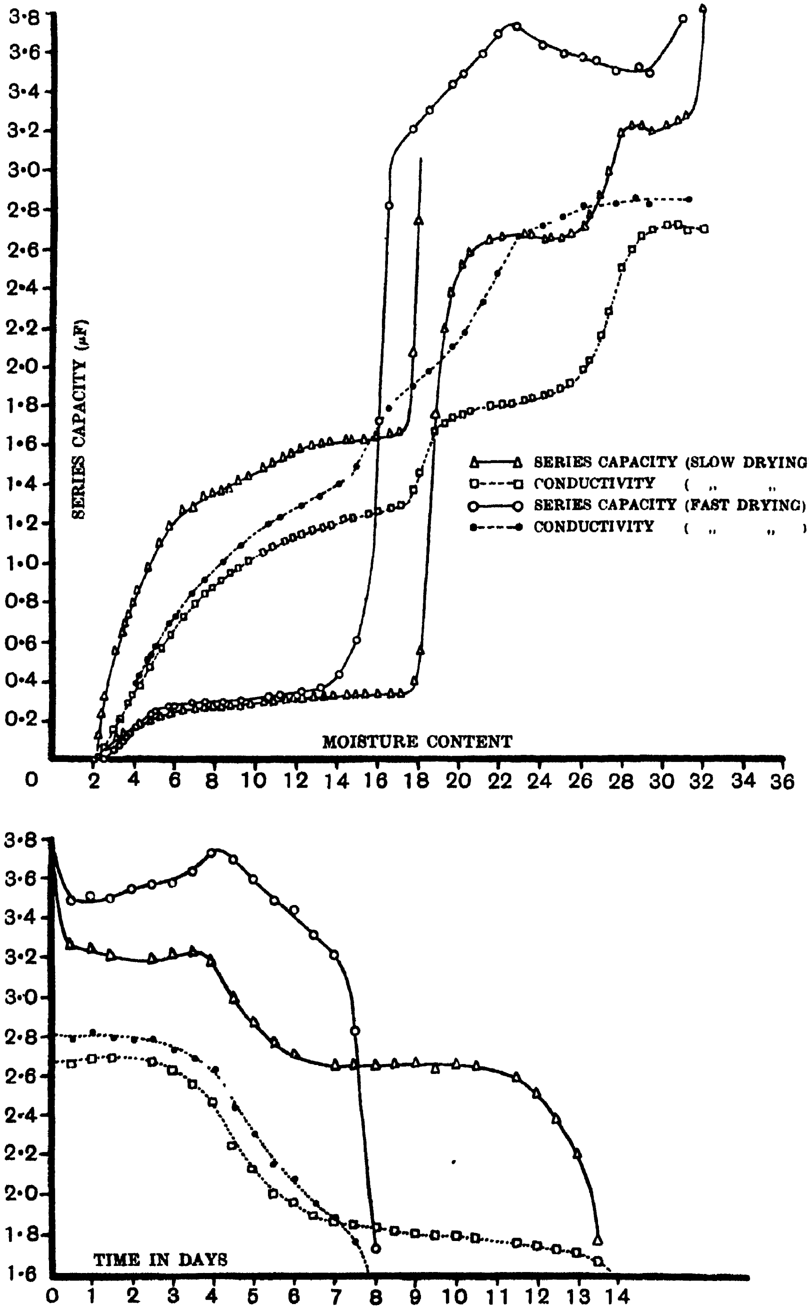
Fig. 4.

measured, and in this case it may be assumed that the soil is responsible for the major part of the parallel capacity measured. Similar calculations for the Long Newton clay show that the electrode effect is more marked, especially for the lower moistures. In general, the experimental data suggests that the electrodes cannot be regarded as identical, as it is not always possible to obtain satisfactory derived curves from the readings for different distances apart of the electrodes.

Conductivity curves.

The type of conductivity curve obtained varies with the characteristics of the soil used, and at least, in some cases, on the rate at which the soil is allowed to dry out. In Fig. 4 is shown the conductivity for the Long Newton clay with carbon electrodes for the faster rate of drying. At the beginning of the experiments there is often a rise in conductivity, due most probably to salt dissolving out from the soil, and consequently if loss of water causes the conductivity to decrease there will be two opposing factors in operation. In the curve shown, the conductivity has a maximum at 29 per cent. moisture, but for the slower rate of drying the maximum occurs at 33 per cent. If the conductivities are plotted against time, it is found that both maxima occur after 4 days, and that the curves agree for a period of 8 days; at this stage the drier block has reached a moisture content of 26 per cent. and the curves diverge. After the maximum has been reached the conductivity decreases with decreasing moisture until the point is reached where air enters the soil pores when the conductivity falls more rapidly. The slow change over the saturated region corresponds to Haines' observation on the constancy of the conductivity of this soil over the same moisture region. The fact that his experiments were completed in a shorter time would help to give a flatter appearance to the curve. After the entry of air the curve is more or less concave to the moisture axis, but is not smooth.

The Punjab soil gives an entirely distinct kind of variation with moisture, the results for the slow and fast rates of drying being shown in Fig. 5. As these curves are for mercury electrodes the series capacities are also given. For the slower drying the conductivity first passes through a maximum and then falls rapidly. This fall is peculiar in that it is steepest at a high moisture and gradually slows down until a moisture of 22 per cent. is reached, the shape of the curve suggesting that the conductivity tends to reach a constant value. At 22 per cent. moisture it commences to fall again, until at 19·5 per cent. there is a sharp drop corresponding with the fall in series capacity. Below 17·4 per cent. the curve is concave


3·8
3·6
3·4
3·2
3·0
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2·6
2·4
2·2
2·0
1·8
1·6
1·4
1·2
1·0
0·8
0·6
0·4
0·2
0
SERIES CAPACITY (μF)
SERIES CAPACITY (SLOW DRYING)
CONDUCTIVITY („ „)
SERIES CAPACITY (FAST DRYING)
CONDUCTIVITY („ „)
MOISTURE CONTENT
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36
3·8
3·6
3·4
3·2
3·0
2·8
2·6
2·4
2·2
2·0
1·8
1·6
TIME IN DAYS
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14

Fig. 5.

to the moisture axis. With carbon electrodes and the slower rate of drying, the general appearance of the curve is similar except that the change at 22 per cent. moisture is not so marked and the decrease between 19·5 and 17·4 per cent. is absent. This shows that the latter effect is characteristic of the mercury electrode, and suggests that the change at 22 per cent. is also an electrode effect. Both changes disappear in the curve obtained by plotting the reciprocal of the difference in series resistance for the mercury electrodes at different distances apart. The initial rise and fall is still present, and as they also occur with the carbon electrodes, they must be due to the soil.

For the faster rate of evaporation the conductivity does not decrease so rapidly, and from a comparison of the two rates of drying it is obvious that time is again an important factor. The conductivity-time curves and series capacity-time curves agree for a period of 6 days, although the block which was drying the faster had lost one-third of its original moisture content and about twice as much as that lost by the other. Consequently for a considerable range below the sticky point ageing effects are of greater importance than moisture in causing variations in the conductivity and capacity. With the faster rate of drying no change similar to that at 22 per cent. moisture for the slower rate of drying can be detected, because the ageing process is not complete and the apparent variation of conductivity with moisture is still considerable.

There is a second difference between the slow and fast rates of drying in that the moisture content at which air enters the pores is greater for the former. This means that the soil particles are in closer packing for the block which dried the faster, and such a difference in packing might arise if the internal friction, which tends to prevent the relative motion of the particles, became greater the longer the soil is in contact with water. Similar effects were obtained to a less degree with a Broadbalk and a Woburn soil, and in all cases the differences between the initial moistures of the blocks of the same soil were quite small. There is, however, the possibility that the blocks were not in the same state of packing at the beginning of the experiment and, in the absence of density measurements, this possibility cannot definitely be ruled out, although the differences in the conductivities do show that ageing effects are taking place. Before a full explanation of the latter can be given it would be necessary to have a clearer picture of the way in which conduction takes place, since a material like soil will exhibit surface conductance. The relative importance of this as compared with the conductivity of the soil solution in bulk can only be determined by measurements made with different concentrations of salt solutions, and a series of measurements with the frequency and voltage of the oscillator varied is desirable.

The bearing of this work on other soil properties will now be considered.

Cohesion, shrinkage and evaporation.

In studies on the forces which bind soil particles together several methods of measurement have been employed: the crushing strength and transverse breaking load of soil cylinders and the resistance offered by soil to the penetration of a wedge have been taken as a measure of soil cohesion. The results depend on the method of preparation of the soil samples and the technique employed, but if the crumb structure of the soil has been destroyed by puddling with water and the soil particles brought into still closer contact by allowing the test samples to dry out, then it is found that the cohesion of most soils increases with decreasing moisture content. This is true of the results obtained by Atterberg and Johansson and by Haines, but, as recorded in the introduction, Atterberg and Johansson's curves were divided into two parts, while those of Haines were continuous. Haines considcred his experimental data was never of sufficient accuracy to exclude definitely the possibility of a break, while the deviations in Johansson's values necessitated his using shrinkage data to define its position with greater accuracy. The way in which the wedge of the Atterberg apparatus penetrates any soil block depends on its moisture content, as when wet the soil shows a smoothly cut surface, and when dry breaks irregularly after a very slight penetration by the wedge. It is known that the breaking load for most materials increases with rate of load, and it was thought that a slow and fast rate might have different relative effects on the drier and moister parts of the curve, suggesting that the disagreement between Atterberg's and Haines' results was due, at least in part, to a difference of this kind. Tests were made on soils with rapid and slow rates of load, but in no case was a break of the magnitude of those of Atterberg obtained. For the fast rate of load a smooth curve could be drawn fitting the points with reasonable accuracy, but when the logarithm of the cohesion was plotted against the logarithm of the moisture content, the curve could be divided into three parts. The first break was that of Atterberg, while the second, which could not always be clearly located owing to experimental inaccuracies, occurred at a moisture of 6 to 9 per cent.

Recently a paper has been published by Christensen (9) on the stress-strain relationships for compression tests for soils at varying moisture content. The maximum bearing strength of the samples was also determined for varying moisture contents and was found to decrease with increasing moisture. Christensen's own description is as follows: "The results representing the mean of three tests seem to follow a definite trend, suggesting an exponential law of decrease approaching the horizontal axis (*i.e.* moisture axis) asymptotically. The equation $p = p_0 e^{-k\omega}$ was therefore used as a basis for smoothing the data. It was first transformed into the logarithmic form

$$\log p = \log p_0 + \log (e^{-k})\,\omega,$$

and an auxiliary graph was made of the latter equation, which showed in some cases a tendency to break into a pair of straight lines."

Thus Christensen's data tends to give a curve of two parts similar to that of Atterberg, and from the graphs given it would appear that such a curve would fit his experimental points better than the exponential curve he has used. There are not enough points for the lower moistures to show if there is the possibility of a break occurring here.

Haines has shown that the shrinkage curve for a soil can be divided into two main portions. In the first, before air can enter the soil pores the volume decrease is equal to the volume of water lost, while in the second, when air is entering the soil, the rate of decrease with loss of water is much less. Haines records that the latter portion of the curve, which represents the residual shrinkage, bends again before reaching zero. As a rough representation of the actual processes taking place in shrinkage, Haines supposed that the residual shrinkage depends on the presence of colloidal material around the soil particles. This forms small pads between them when they have been brought into contact by the first stage of the shrinkage, and only begins to lose water when air is replacing water in the soil pores.

The shrinkage and cohesion curves for the Punjab soil are shown in Fig. 6. It will be seen that the shrinkage curve agrees with the results obtained in the present experiments, the change from the main to the residual shrinkage commencing at 19·5 per cent. moisture, and the bend in the residual shrinkage occurring at 8·5 per cent. moisture. The cohesion curve is that for a slow rate of load and gives the Atterberg break at 20 per cent. moisture, and a second break at a moisture of the order of 9 per cent. The three readings for the lower moistures are not accurate, but they definitely lie off the second part of the curve.

There is one other type of experiment in which discontinuities have been observed—the evaporation of water from a puddled soil. When the rate of evaporation is plotted against moisture content, then under certain experimental conditions a curve is obtained which consists of three parts, the variation with moisture being nearly linear in each. Fisher (10) suggested that the ease of movement of water from the inside of the soil to the outside was a limiting factor, and that this was the explanation

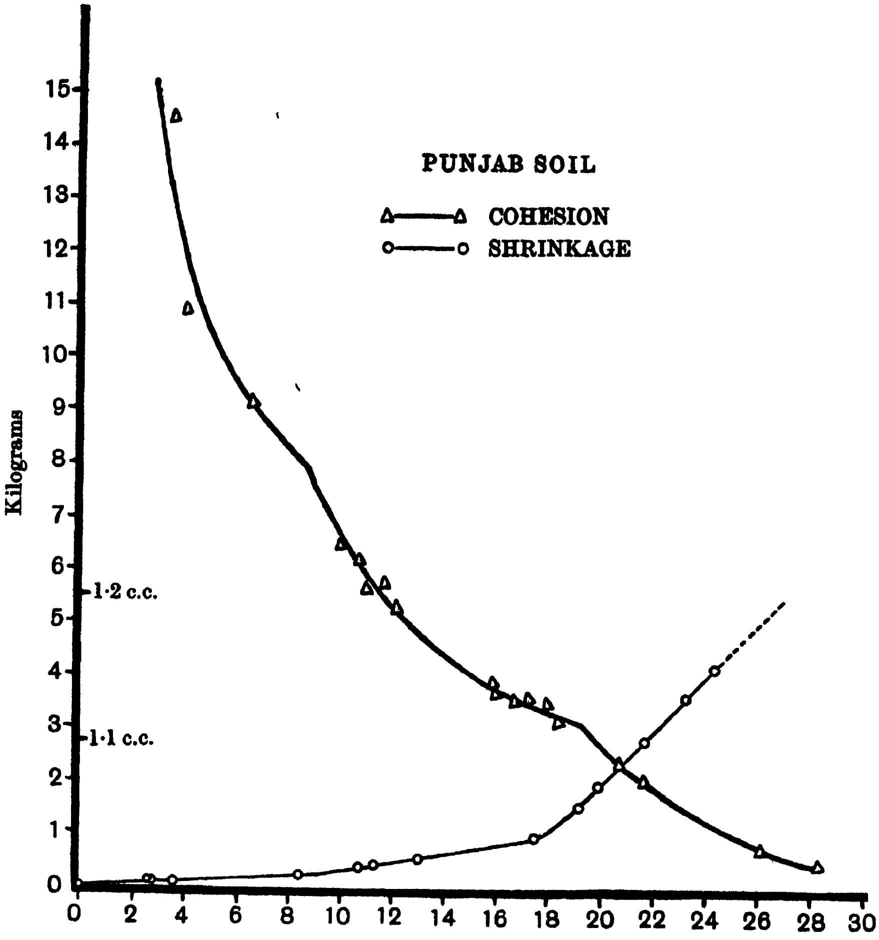

Fig. 6.

for the discontinuous rate curves he usually obtained. The rate of evaporation depends on two main groups of factors, the soil itself and the environmental conditions, and as shown by Keen, Crowther and Coutts(11), the kind of rate variation with moisture obtained depends very largely on their relative importance. With soil in inverted weighing bottles, bulk-air movements were reduced, and the rate of evaporation was low and constant down to low moisture contents. What was really being measured was the rate of diffusion of water vapour from the soil surface to that of the acid, and soil factors did not become apparent until low moistures were reached. When the soils were supported below glass plates the curves obtained varied with the area of the plate covered. A rate curve consisting of three nearly linear portions was obtained for the plate completely covered with soil, the authors concluding that this type of curve was the resultant of two dominant and opposing forces, a more rapid evaporation from the outer edges of the plate and the water movements outward by capillary action as water gradients are set up. Thus when the soil tends to become the controlling factor the moisture range is divided into three parts similar to those observed for the other soil properties described in this paper.

SUMMARY.

1. The equivalent parallel capacity and conductance of a series of soil blocks have been measured for decreasing moisture content.

2. The results obtained depend on the electrodes used. With mercury, all soils give curves of the same general type for the variation of the capacity with moisture because the capacity effects associated with the soil electrode interface are large compared with those due to the soil. The electrical properties of the interface have been shown to exhibit marked changes in their variation with moisture at certain moisture contents.

The results obtained with carbon electrodes, though depending on the texture of the soil, generally confirm the changes observed with the mercury electrodes.

3. Of the four characteristic moistures found, the second is readily identified as Atterberg's "Schwindungsgrenze" (the moisture at which air enters the pores), while the first appears to correspond with his "Ausrollgrenze" (the lower plastic limit).

The two lower moistures are hitherto unrecorded, but there are indications that at the lower one changes may occur in the variation with moisture of the shrinkage and cohesion of soil, and the rate of evaporation of water from soil.

In conclusion, I wish to thank Dr R. K. Schofield for much helpful advice during the course of this work.

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THE PRESENT POSITION OF THE THEORY OF THE COAGULATION OF DILUTE CLAY SUSPENSIONS.

A RÉSUMÉ.

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I. INTRODUCTION.

THE object of this paper is to review the present position of our knowledge of the coagulation of dilute clay suspensions in the light of the electrical theory of colloidal behaviour. In recent years an intensive study has been made on the behaviour of a few typical colloidal suspensions, but up to the moment no such intensive study on the behaviour of dilute clay suspensions has been made. But many of the results found for other systems apply to clays, so that it is possible to deduce from studies of other systems properties that clay systems will be expected to possess. The electrical theory can hardly be said to have been originated by any one person, since it has been constantly altering, though Quincke and Helmholtz, and in this country Hardy and Whetham, were probably the first to give it definite shape. It has since been developed by many workers, of whom Smoluchowski, Freundlich, Pauli, and Wiegner may be named as examples. But it still suffers from one great disadvantage that it is not yet a definite theory. There are no generally accepted foundations to the theory, nor is it universally agreed what physical principles should be used in its development. Further, it is handicapped by the fact that if one does decide on a particular hypothesis, no rigorous consequences can in general be drawn from it as the mathematical methods available do not appear to be powerful enough to make any exact deductions. In consequence, a confusion has often arisen between the separation of the physical approximations and vaguenesses in the assumed theory on the one hand and the mathematical and physical approximations that must be made in deducing the consequences of the theory, due to the lack of adequate mathematical methods on the other.

In this paper, then, an attempt will be made to see how far the properties of clay suspensions can be accounted for by this electrical theory, and inversely what type of theoretical knowledge can be gained from a given type of experimental data. As the title of this paper indicates, a limitation will be observed on the types of systems considered. Only dilute clay suspensions, that is only suspensions in which the volume of the clay particles is small in comparison with the volume of the whole system, will be discussed. This limitation is necessary, since it is only very recently that any systematic work has been started on the properties of more concentrated suspensions, and even now there appears to be no type of mathematical analysis that will be powerful enough to deduce even the simplest properties of such systems. Further, a complication arises that once the suspension contains more than a certain concentration of clay, it will show rigidity under certain conditions, due to the individual clay particles not being independent of one another, but forming some kind of linked structure throughout the whole system. This effect will not be considered here at all, but reference can be made to the recent work of McDowell and Usher (1931) who have begun a systematic investigation on it. The following discussion then will only be valid for clay suspensions containing not more than 0·1 per cent. by weight of clay, though 0·01–0·5 per cent. is the range of concentrations usually employed. The second limitation that will be observed is that only clay suspensions, and not soil suspensions, will be discussed. This is because most of the earlier work on soil suspensions produced data unsuitable for exact treatment, and although it is possible to offer explanations of most of the results obtained by assuming various conditions which may have been present, such discussions are in general unconvincing. The whole problem of the coagulation of heterogeneous systems, such as soil suspensions, should be re-investigated before an exact discussion of their properties can be profitably undertaken.

II. THE RATE OF COAGULATION OF SUSPENSIONS.

Rapid perikinetic coagulation.

Coagulation is the name given to the process in which the number of particles per unit volume of a sol diminishes by their adherence one to another, so that the rate of decrease of the particle numbers per unit volume of a sol is the fundamental quantity to be measured experimentally in coagulation phenomena. From these studies it will be shown what conclusions can be drawn about the mechanism of coagulation and the properties of the suspended particles themselves. To arrive at a quantitative theory for the rate of coagulation two separate sets of factors must be discussed, namely those which produce the collisions between the suspended particles, and those which cause the particles to adhere to one another during a collision. The first set of factors will be considered in this and the third subsection, while the second set will be dealt with in the second subsection.

The first step in discussing the factors influencing the rate of collisions between particles in a sol is to define what is meant by a collision. Supposing the particles are uncharged and exert no forces of any kind on one another, a collision occurs between two particles when their surfaces touch. But if the surface is not definable owing to surface hydration or other causes, or if the particles are charged so as to exert attractional or repulsional forces on one another, then a collision will be said to occur whenever the centre of gravity of the two particles approach within a definite distance of one another, which may depend on their relative orientations and energies.

Three quite different processes can cause suspended particles to collide with one another, namely:

(1) The particles may attract one another by virtue of their electrical properties.

(2) The Brownian motion of the particles. Following Wiegner (1928), collisions produced by this process will be called perikinetic collisions, for the colliding particles move in no preferential direction, they move in all directions equally freely.

(3) The mass motion of some particles relative to others. Such collisions will be called orthokinetic collisions, as there is at least one direction singled out in which the colliding particles move. Such mass motion can be caused by one group of particles settling under gravity through another, or moving through another in a centrifugal or electrical field, or even by the dispersion medium having a varying mass motion, as for example if it is flowing down a capillary tube, in which case a velocity gradient is set up between the wall and the centre of the tube.

In 1916 Smoluchowski published his classical paper on the rate of coagulation of sols. He started with the observed fact that when increasing quantities of electrolyte are added to a lyophobic sol, as for example a gold sol, the rate of coagulation increases until it reaches a maximum, which is independent of the coagulator used. When the coagulation is proceeding at this maximum rate it is called a rapid coagulation, and it is only with such a coagulation that he dealt. His fundamental hypothesis was that, when a sol is undergoing rapid coagulation every collision between two particles results in their sticking together, and remaining together during the rest of the coagulation, no hypothesis of any description being necessary concerning the causes of the adhesion. Further, he only contemplated perikinetic collisions, namely those caused by the Brownian motion of the particles. Thus he developed his theory subject to the following conditions:

(1) All the particles initially present in the sol are spherical and identical with one another.

(2) The effect of all external directional forces on the particle's motion is negligible and that there is no differential mass motion of the liquid, *i.e.* the coagulation is purely perikinetic.

(3) No particle exerts any force on any other particle outside a definite sphere of radius A, called the attractional radius of the particle drawn around it. But whenever the centre of a second particle approaches within this distance of the first, these two particles stick together and do not become unstuck subsequently during the coagulation. That is, the coagulation is rapid.

In order to solve his mathematical equations he had to make three approximations, that later mathematical analysis has not yet been able to remove, namely:

(4) The sol is so dilute that ternary and higher order collisions between the particles can be neglected, and that the volume occupied by the particles is small compared with the volume of the sol.

(5) The complex particles resulting from the sticking together of two or more primary particles have the same hydrodynamical constants as the primary particles. In particular if A is the radius of attraction of a primary particle and D its diffusion coefficient in the dispersion medium, and A_{ij} is the attractional radius of a secondary particle containing i primary particles, for a secondary particle containing j primary particles, and D_i and D_j are the diffusion coefficients of these two secondary particles, then he assumes

$$2AD = A_{ij}\,(D_i + D_j).$$

This is the really weak assumption in the theory.

(6) The formula is only valid after the coagulation has been proceeding for a certain time t, which must be large compared with A^2/D. For a usual colloidal sol, however, it implies that t must be greater than 10^{-4} sec. Under these conditions Smoluchowski showed that for a sol containing initially ν_0 particles per cubic centimetre, the number ν_t remaining t seconds after the beginning of the coagulation is given by

$$\nu_t = \frac{\nu_0}{1+\beta t}, \text{ where } \beta = 4\pi D A \nu_0.$$

Further, if the particles are assumed to be sufficiently large, so that the resistance of the dispersion medium to their motion is given by the Stokes' law formula, then

$$D = \frac{kT}{6\pi\eta\rho},$$

where T is the absolute temperature of the sol, k is Boltzmann's constant, equal to $1{\cdot}372 \times 10^{-16}$ ergs per molecule, η is the viscosity of the dispersion medium, ρ is the radius of the particle, calculated from the Stokes' law formula.

Using this value for D, the above formula reduces to

$$\nu_t = \frac{\nu_0}{1+\beta t},$$

where

$$\beta = \frac{2kT\nu_0}{3\eta}\frac{A}{\rho}. \qquad \text{......(1)}$$

The only quantity that cannot be measured independently of this equation is A, but in general ρ is difficult to determine, so that the ratio A/ρ is the quantity usually determined, for ν_t, ν_0, η, T can all easily be evaluated. The value of A/ρ can under certain conditions be assigned *a priori*, for if the particles are assumed to have a definite surface, and if two particles only adhere to each other when their surfaces touch, then $A = 2\rho$, so that $A/\rho = 2$.

This formula (1) has been abundantly verified by subsequent experimenters using more or less lyophobic sols, and A/ρ always comes out about 2. For present purposes the works of Wiegner and Tuorila (Wiegner and Tuorila, 1926; Tuorila, 1926 and Wiegner, 1928) will be the most convenient to present, owing to the large amount of uniform accurate data they have obtained. These authors used gold and paraffin sols, and clay and kaolin suspensions. The gold sols were very nearly monodisperse and probably consisted of nearly spherical particles, while the other sols and suspensions were not so well graded, and probably for the clay suspension at least, had not such spherical particles. The course of the coagulation was followed in detail under the ultramicroscope, and the particle density ν_t was determined directly after different intervals of time, thus A/ρ could be computed. The main results of this investigation were that the quantity β of formula (1) remained practically constant during coagulation, although it showed a slight tendency to decrease as the coagulation proceeded, indicating a slight slowing up in the rate. Further, it was independent of the nature of the particles, being the same for gold, kaolin and clay, and of their size, being independent of ρ. Marshall (quoted by Wiegner, 1931) showed for gold sols A/ρ was independent of the dispersion medium, being the same for a gold sol dispersed in alcohol as one dispersed in water, and Garner and Lewis (1926) and Butler (1930) showed A/ρ was independent of the temperature of the sol. Thus formula (1) appears to be valid for all the systems so far examined.

The extension of Smoluchowski's theory to the rapid coagulation of monodisperse sols containing non-spherical particles presents a large number of mathematical difficulties which have only partially been overcome. The probability that two non-spherical particles in a suspension will collide with one another is greater than the corresponding probability for two spherical particles due to:

(1) The increased number of degrees of freedom possessed by a non-spherical particle capable of bringing about a collision. A spherical particle has only three translatory degrees of freedom that can bring about collisions, but non-spherical particles have up to three rotational ones that can become effective.

(2) The attractional surface around a non-spherical particle of a given volume is greater than that around a spherical one of the same volume.

Experiments of Wiegner and Marshall (1929*a*) showed that the rapid coagulation of needle-shaped particles proceeds initially very much faster than for spherical particles, and this effect is the more marked the more concentrated is the sol, but that the rate decreases as the coagulation proceeds owing to the secondary aggregates becoming more spherical. Müller (1928*b*) was able to make some approximate calculations on the increased rate of coagulation for needle-shaped and disc-shaped particles due to their increased attractional surface compared to spherical particles. But his results for needle-shaped particles come out too low, showing that he has not been able to account for the whole of the increased rate.

More headway, however, has been made with the extension of Smoluchowski's theory of rapid coagulation to polydisperse systems containing spherical particles, that is to systems whose particles are spherical but of various sizes. Müller (1928*b*) proved very generally that a polydisperse system undergoing rapid coagulation, coagulates faster than a monodisperse system containing the same number of particles initially, which result had previously been found experimentally, and that a slight symmetrical polydispersity in an almost monodisperse sol about the mean particle size, was almost without influence on the rate of coagulation. But he worked out the particular case (Müller, 1928*a*) of the coagulation of a mixture of two monodisperse sols much more completely, and was able to calculate the total number of particles present at any time *t* after the beginning of the coagulation. He had to make all the assumptions that Smoluchowski made and a few others, so his formulae are unfortunately only very approximate. Wiegner and Tuorila (1926), Tuorila (1926) and Wiegner (1928) tested these formulae experimentally, using a series of monodisperse gold sols, and showed that:

(*a*) If the ratio of the radii of the larger to the smaller group of particles lies between 1 and 2, the course of the coagulation is similar to that for a monodisperse sol.

(*b*) If the ratio of the radii lies between 10 and 30, and if there are a large excess of smaller particles over large, then the course of the coagulation differs definitely from that for a monodisperse sol, but follows Müller's formula within the experimental error.

(*c*) If the ratio of the radii lies between 30 and 60, the coagulation appears to proceed slightly slower than is given by the Müller formula. It is a little difficult to explain this divergence, for so many approximations have to be made in the mathematical theory. Further, Wiegner and Russell (1930) showed that the counting of the particle numbers in very polydisperse systems may be liable to considerable systematic error. Hence it is not yet possible to be sure of the true magnitude of the divergence, nor to determine what factors it is due to.

Thus the main result that emerges from this subsection is that when rapid coagulation is taking place, whenever two particles touch one another they stick together, and remain together during the rest of the coagulation. The collisions between particles are brought about solely by the Brownian motion of the particle, and no recourse is necessary to any other attractional forces between particles. But nothing is said about the adhesional forces between the particles except they are practically surface forces.

Slow perikinetic coagulation.

Turning now from the rapid perikinetic coagulation of sols, when the theoretical results can be accurately predicted whenever the mathematical difficulties can be overcome to the slow perikinetic coagulation of sols, it will be seen that in general the theory is as yet only very imperfectly understood. Recapitulating, it will be recalled that the rate of coagulation depends on two probabilities, namely the probability of collisions between particles and the probability of adhesion between particles whenever they collide with one another. When a sol is undergoing slow coagulation, the particles in general carry an electrical double layer outside their surfaces, so that they will exert powerful forces on one another whenever they approach very close. These forces are such as only to alter the space and velocity distribution of the particles slightly, so that they will not appreciably affect the probability of collisions between particles, but only the probability of adhesion between them. Hence some definite hypothesis will have to be introduced stating under what conditions two particles will adhere to one another in a collision. But at the outset two different types of slow coagulation must be distinguished. When a sol is coagulating very slowly, the particles often do not stick together at random points on their surfaces, but only along definite axes, so that a particle may act analogously to a centre of crystallisation for other particles, which will orient themselves so that the new complex aggregate possesses an ordered structure. Wiegner and Marshall (1929*b*) gave a good example of this phenomenon, which is usually called the ageing effect of sols, in the slow coagulation of vanadium pentoxide and congo rubin sols. When these sols, whose particles were initially approximately spherical, underwent very slow coagulation, the secondary aggregates found were very long, thin, needle-shaped particles. If, on the other hand, the sol is coagulating fairly rapidly, then the secondary aggregates formed possess less-marked ordered structures, and eventually reach a stage when they possess a loose unordered structure, similar to those produced by rapid coagulation.

Smoluchowski (1917) put forward a theory for the rate of coagulation of a monodisperse sol undergoing slow coagulation. He assumed that the number of collisions a particle suffers in unit time is the same as if the sol were undergoing rapid coagulation, but that the probability of adhesion due to a collision, instead of being unity, now has a value ξ. That is, in only a fraction ξ of the collisions between particles do the particles stick together; in the other fraction of the collisions they come apart again. His formula for the rate of perikinetic coagulation in a monodisperse sol containing only spherical particles is

$$\nu_t = \frac{\nu_0}{1+\beta\xi t},$$

where

$$\beta = \frac{2kT\nu_0}{3\eta}\frac{A}{\rho}, \qquad \text{......(2)}$$

where, as before, ν_t is the number of particles remaining per unit volume of the sol, t seconds after the commencement of the coagulation. Freundlich (1918) gave a physical interpretation to ξ by assuming that the colloidal particles undergoing slow coagulation repel each other, and that for two particles to stick together their total kinetic energy must exceed the energy required to overcome their mutual repulsive forces. Thus he assumed that whenever two particles having the sum total of their kinetic energies greater than some critical value collided, then the particles adhered to one another.

This formula of Smoluchowski's has been examined by various authors, and their results seem to show that for strongly hydrophobic sols, ξ remains practically constant during the coagulation, but as the sol becomes hydrophilic ξ decreases during the coagulation, this decrease being the more marked the slower the coagulation. Thus Tuorila (1926) and Scherf (quoted by Wiegner, 1928) showed that for monodisperse gold sols ξ remains constant during the coagulation, and Tuorila (1928 *b*) showed that the same is true for a paraffin sol also unless the coagulation is very slow, when ξ decreases as the coagulation proceeds. Kruyt and van Arkel (1923) and Kruyt and de Haan (1930) showed that ξ decreases for a selenium sol quite markedly, while Tuorila (1928 *b*) showed that for a clay suspension ξ decreases so rapidly during the coagulation that the Smoluchowski formula possesses no theoretical value whatever for specifying the rate of such a coagulation.

The reasons for the failure of the Smoluchowski formula are still very uncertain. There seems no adequate grounds for questioning the validity of the assumption that the number of collisions between the particles is that given by the simple kinetic theory. Possible reasons for this failure may be:

(1) ξ really does decrease with the time. Kruyt has developed this view and has assumed that the colloidal particles are not uniformly charged over their whole surface. If when two particles collide the points of contact are uncharged, or very slightly charged spots, the two particles will stick together. As coagulation proceeds the complex particles possess an increasing proportion of highly charged spots on their surfaces, for the uncharged spots get covered with other particles, so the complex becomes more stable, and consequently coagulates slower. Kruyt and de Haan (1930) measured the electrokinetic potential of the particles, and showed that it does increase slightly during coagulation, thus supporting their contention.

(2) That collisions between complex aggregates can result in their partial break-up, so that although ξ, the probability of adhesion, remains constant, a new probability enters giving the probability of break-up of the complex in a collision, so the rate of diminution of particle numbers

is not determined by ξ alone. This would be in accord with the fact that it is precisely reversible colloids, *i.e.* colloids which can be redispersed after coagulation by simple dilution, which show this slowing up in the rate of coagulation most markedly.

(3) The sols used were not truly monodisperse, so the formula (2) is inapplicable. Tuorila (1926) showed that if a bidisperse gold sol, *i.e.* a sol containing only two sizes of particles, is undergoing slow coagulation, the larger particles coagulate faster than the smaller, so that as coagulation proceeds, its rate becomes slower, since it is increasingly controlled by that for the small particles. He suggested (1928 *b*) that the cause of the slowing up in the rate of coagulation of clays is to be found in the polydispersity of the clay suspensions used.

In support of this third effect Müller (1928*a*) showed theoretically that, using the theory of the critical potential (*vide infra*, p. 188), this effect should be expected, for with a given concentration of electrolyte in a sol of a given material, the electrokinetic potential of the particles decreases as their radius increases, so that the larger particles should coagulate faster than the smaller, as observed by Tuorila. Some recent work of Burton and Annetts (1931) also probably lends some support to this effect. They found that if a small quantity of electrolyte is added to a sol (they used gold, arsenious sulphide and mastic sols) so that it undergoes slow coagulation, a certain proportion of the particles seem to undergo complete coagulation and sediment out. If more electrolyte is added, more particles will suffer complete coagulation, and so on. They further showed that coagulation began when the electrokinetic potential of the sol, as measured by the U-tube method, was very high. They state that they consider this is unlikely to be a polydispersity effect, as centrifuging the sol does not destroy it. However, Tuorila (1926) showed that this effect does not exist for a monodisperse gold sol, and as Burton and Annetts found it with a polydisperse gold sol, it is not improbably due to the polydispersity of the sol. According to Müller's theory, in which the electrokinetic potential of particles in the presence of electrolytes depend on their size, they would only have measured the electrokinetic potential of the smallest particles, *i.e.* those having the largest electrokinetic potential, by their U-tube method, and this could be much larger than that of the larger particles.

The three possible reasons for the failure of the Smoluchowski formula are obviously not mutually exclusive, and it is possible that they are, as a matter of fact, all operative, although it is not yet possible to separate out their relative importance in any particular case. Thus in concluding this section on the rate of slow coagulation of sols, it is seen that a quantitative theory can only be given for markedly hydrophobic sols coagulating not too slowly. The details of the processes involved in slow coagulation for more hydrophilic sols are not yet known.

Orthokinetic coagulation of suspensions.

Orthokinetic collisions between particles are defined as those produced by the mass motion of one group of particles relative to another, and if some of the particles stick together as a result of such collisions, they are said to be undergoing orthokinetic coagulation. The most important type of orthokinetic collisions are those produced by larger particles settling under gravity through a suspension containing smaller, or at least slower settling, particles. The collisions can be elastic, in which case no coagulation is taking place, or a certain fraction can result in the production of more complex particles, in which case slow coagulation is taking place, or all the collisions can produce more complex particles, in which case rapid coagulation is taking place. Up to the present, very little work has been done on this type of coagulation, but the preliminary theoretical work of Tuorila (1927) and Müller (1928*b*) and the experimental work of Tuorila (1927) and Wiegner (1928) give results from which one can form some estimate of its importance in any particular instance. Tuorila gave an approximate solution for the problem of a uniform group of spherical particles sedimenting through a uniform group of smaller spherical particles, by the introduction of the concept of the "Hautraum" of a larger particle. The "Hautraum" of a larger particle is defined, in this particular case, as the volume of the suspension from which all the smaller particles are removed per second by it, assuming that rapid coagulation is taking place. Thus his analysis only contemplates the following problem:

(1) The suspension is undergoing rapid coagulation.

(2) The suspension is bidisperse, the particles are spherical, and obey Stokes' law. Further, the number of the larger particles per unit volume is so small that they do not interfere with each other hydrodynamically, nor affect the distribution of small particles around one another. This last proviso is to allow the distribution of small particles around a larger one to depend only on the time since the commencement of the coagulation, and not on its position.

(3) The total particle density is less than 10^8 per cubic centimetre, so that perikinetic coagulation is negligible. In order to formulate and solve his mathematical equations he had to assume:

(*a*) when a small particle coagulates with a larger one, the new particle so formed acts as a sphere whose volume is the sum of the volumes of the two particles;

(*b*) the "Hautraum" b of a larger particle is given by

$$b = \pi (A^2 - R^2)(v_R - v_r),$$

where $A = R + r$ and R and r are the radii of the larger and smaller particles respectively, and v_R and v_r are their sedimentation velocities.

If initially there are N_0 large particles per cubic centimetre of the suspension and n_0 small ones, then the number of small particles n_t present per cubic centimetre of the suspension t sec. after the beginning of the coagulation is

$$n_t = n_0 e^{-N_0 B_0 t}, \qquad \ldots\ldots(3)$$

where B_0 is a constant, and where r/R and m/M lie between 0 and 0·4. Here m is the total weight of the small particles and M of the large particles initially present per cubic centimetre, both groups of particles being assumed to have the same density. Tuorila, using an approximately bidisperse quartz suspension, did in fact show that B_0 remains constant during the coagulation and was independent of the number of large particles initially present. Müller improved on Tuorila's analysis, particularly with regard to his second approximation (*b*) above. He evaluated the "Hautraum" from strict hydrodynamical principles, assuming that the liquid is incompressible and that there is no slip between the particle's surface and the liquid. But his equations are very complicated and can only be solved by numerical integration, and are only valid for the initial rate of decrease of particle number. His analysis showed that Tuorila's results give a fair approximation to the more accurate solution.

Tuorila extended this work to the explanation of some of the rather curious phenomena met with in flocculation and coagulation studies in soils and clays. It will be convenient to introduce here a distinction between coagulation and flocculation. A suspension has been defined to be undergoing coagulation if the number of particles per unit volume decreases by their sticking together. It will be said to flocculate when the secondary aggregates so formed settle in flocs, and it will be said to be completely flocculated when these flocs settle, leaving a clear supernatant liquid above them. Tuorila virtually discussed under what conditions flocculation, and in particular complete flocculation occurred. For example, if the larger particles are much larger than the smaller, *e.g.* ten times larger, they would sediment through the suspension without carrying down an appreciable fraction of the smaller. Similarly to get complete flocculation not only must the large particles be not too large, but they must be sufficiently numerous to be able to clear the suspension. This explains why it is so difficult to flocculate a very dilute clay suspension completely, for there would not be sufficient of the larger aggregates to carry down the smaller ones.

In concluding this section, it will be recalled that the velocity of coagulation of a sol or suspension depends on two probabilities, the probability of collision between particles, and the probability of adhesion between them in a collision. It appears that the physical phenomena are sufficiently well understood for the first probability to be accurately evaluated whenever the mathematical equations can be solved. Further, it yields surprisingly little knowledge about the particles themselves. The second probability cannot in general be accurately evaluated, for here the physical foundations are very imperfectly understood. In the succeeding sections some of the theories concerning the detailed structure of the colloidal particles will be reviewed, and it is only from such considerations that the factors determining the probability of adhesion can be ascertained.

III. THE ELECTROKINETIC POTENTIAL.

The electrical double layer.

In the preceding section it was shown that the rapid coagulation of sols is due to the suspended particles colliding with each other due to their Brownian and other superimposed motions, and that the particles themselves behaved as uncharged. But if the sol is only coagulating slowly, a probability of adhesion ξ must be introduced, to be determined *a posteriori* from the experimental data, and which is dependent on the amount of electrolyte or other soluble substances present with the dispersion medium. But before this subject can be further developed, the electrical properties of the suspended particles themselves must be examined. In this section the electrical potential of the particles will be discussed.

In general, when a colloidal particle is suspended in water, or in a solution, it acquires an electrical charge, either because it can effect a differential adsorption of ions from the solution, or because of a differential dissociation of the ions from its surface. Possibly under certain conditions both these mechanisms are operative. This charged surface alters in its neighbourhood the distribution of the ions in the solution, thus setting up an electrical potential between it and the bulk of the solution. The properties of this potential were first examined by Helmholtz in 1879. He assumed, for mathematical convenience, that the particle's surface carried a uniform surface charge which was neutralised by a uniform oppositely charged layer at a fixed distance away from it. If the particle is spherical, of radius r, and carrying a charge e, and if the oppositely charged layer is at a distance δ from its surface, then assuming r to be large compared to δ (that is, the double layer around the particle is thin), the potential difference across the double layer is $e\delta/Dr^2$. Helmholtz himself did not insert D, the dielectric constant of the medium in the double layer, into his formula, but Pellat (1904) showed that this formula gave results inconsistent with atomic size then newly determined by Perrin unless the dielectric constant of the dispersion medium is inserted. For a particle suspended in a solution it is generally assumed that the dielectric constant between the layers is the same as that of the dispersion medium in bulk, though there is some evidence against this assumption. Gradually this outer layer became identified with an ionic layer, one ion thick, which was supposed to surround the particle.

This picture was modified in 1909 by Gouy and later by Chapman (1913). They supposed that instead of the outer ionic layer being fixed, the ions move under their own heat motion freely in the solution, but are attracted or repelled by the charged surface according to the sign of the charge they carry. The outer layer of the double layer is thus formed by there being in the neighbourhood of the surface an excess of ions carrying an opposite charge to that on the surface over those carrying a like charge, and, on the average, this excess must be just sufficient to keep the whole system electrically neutral. Debye and Hückel (1923) and Müller (1928*a*) have both improved on the mathematical analysis of this diffuse double-layer theory, which, however, still remains very approximate. The fundamental assumption made in this theory is that the surface of the particle adsorbs no ions at all, and its charge remains constant. The other principal assumptions and approximations made are:

(1) The ions do not influence the molecules of the solvent, which automatically rules out all hydration effects in aqueous solutions.

(2) All salts present are completely dissociated, or at least the ions do not influence each other except in so far as they exert a simple electrical attraction or repulsion on one another.

(3) The simultaneous validity of Boltzmann's and Poisson's equations in the neighbourhood of the surface throughout volumes sufficiently

small to allow the electrical potential to be sensibly constant throughout the volume: that is, the fluctuations of ionic density in such a volume must be small compared with the mean ionic density present in that volume. This is equivalent to assuming that the colloidal surface is so large, and the diffuse double layer so thick, that such a volume will contain an appreciable number of ions. This assumption has been discussed in detail by Fowler (1929).

(4) The ionic density is small, that is, the solution is everywhere dilute. This makes the third assumption much more serious.

The fourth assumption can probably be made comparatively unobjectionable, allowing up to even tenth molar solutions to be considered, if the analysis of Gronwall, La Mer and Sandved (1928) can be taken over from the theory of strong electrolytes. The final results are all very complicated expressions, except one, which is a direct consequence of Boltzmann's theorem. This result states that the distribution of ions around the particle must satisfy a set of conditions identical with the Donnan membrane-equilibrium formulae. Suppose a charged particle ionises to give a cation of type K and valency α, and is in a solution containing the salt formed by these cations with an anion of type A and valency β, then the ionic concentrations $[K]$ and $[A]$ at any two points 1 and 2 outside the particle, must satisfy the relation

$$[K]_1^\alpha [A]_1^\beta = [K]_2^\alpha [A]_2^\beta,$$

where $[K]_1$ represents the concentration of the cations at the point 1. Unfortunately, this result cannot as yet be directly verified.

The fundamental assumption on which the diffuse double-layer theory is based, namely that the surface is uniformly charged and adsorbs no ions, is invalid for many systems. Since 1920 Freundlich has frequently emphasised a qualitative extension of this theory, which was later given mathematical form by Stern (1924), for the case when ionic adsorption is taking place on the charged surface. The picture given is that the oppositely charged ions fall into two classes, those forming the adsorption layer which are tightly bound to the particle's surface, thus forming a double layer of the type contemplated by Helmholtz, and those dissociated from this layer, and any other ions present in the solution, which go to form the diffuse double layer. Using this picture Smoluchowski (1914) showed that it was reasonable to assume that the electrokinetic potential of such a system is the difference of potential in the diffuse double layer alone, while the potential difference between the surface and the bulk of the solution was the thermodynamic potential of Nernst.

Bjerrum (1926) extended the diffuse double-layer theory by preserving its essential assumption but emphasising the important rôle played by ionic association. He showed that the density of ions near a colloidal particle or other charged body depends on the size of the ion and on its valency. For ions carrying a charge opposite to that of the surface, the smaller the ion, the closer it can get to the particle, and the higher will be its mean density in the immediate neighbourhood of the particle. It will, in fact, appear to be increasingly more associated with the particle as its radius decreases. Similarly, the higher the valency of such an ion, the larger is the neighbourhood around the particle in which the ion will appear to be associated with it. An ion is said to be associated with the surface, when the mean time it remains in a small volume near the surface is longer than the mean time it remains in an equal volume in the bulk of the solution. On this theory the electrokinetic potential of the particle is the part of the potential difference due to the mobile or weakly associated ions. This explains why particles containing divalent ions in their outer layer have a lower electrokinetic potential than particles containing monovalent ions, for a larger proportion of the divalent ions are associated with the surface. Similarly, assuming the hydration theory of the alkali ion, it explains why particles having lithium or sodium ions in their outer layer have a higher electrokinetic potential than particles having caesium or rhubidium ions, for the former, owing to their greater hydration, act as larger ions, so cannot approach the surface so closely as the latter, and are thus less strongly associated with it.

The measurement of the electrokinetic potential.

The great weakness in the whole quantitative electrical theory of colloids is the difficulty in measuring the electrokinetic potential of a particle, which is a fundamental property on any such theory. Up to the present there have been no direct exact methods available, so that indirect methods have been used. Four distinct methods have been proposed, two suitable for dilute suspensions of particles and two for large fixed surfaces. So far, only one method has been used for clay particles, namely measuring their mobility or migration velocity in an electrical field, when they are present in a dilute clay suspension, and from this mobility to calculate their electrokinetic potential. Thus two distinct problems are involved, the discovery of a relation connecting the mobility of a charged particle with its electrokinetic potential, and the actual measurement of the mobility itself. The theoretical weakness of this method of measuring the electrokinetic potential is obvious. An equilibrium property of the colloid particle, namely its electrokinetic potential, is being calculated from a so-called transport property, namely its mobility. The analogous problem for strong electrolytes is the calculation of ionic activity from conductance data, which is one of the least satisfactory methods available. Both for colloids and strong electrolytes the mathematical results for the equilibrium problems are comparatively exact, while for the transport problems very approximate.

To solve the first problem a difficulty arises immediately, for there is no *a priori* relation between mobility and electrokinetic potential. Detailed assumptions about the structure of the electrical double layer are necessary. Helmholtz (1879), using his fixed double-layer picture, showed that if a spherical particle has an electrokinetic potential ζ and a mobility u, that is, if it moves with a velocity u cm. per sec. in an electrical potential gradient of one unit per cm., then

$$\zeta = \frac{4\pi\eta u}{D},$$

where η is the viscosity and D the dielectric constant of the dispersion medium. Helmholtz himself did not insert the dielectric constant into this formula, and his whole mathematics was extremely approximate. The first serious attempt to find a similar relation on the diffuse double layer theory was made by Debye and Hückel (1924) and Hückel (1924). Using their theory of strong electrolytes, they showed that a charged particle in an electric field will be acted upon by a force proportional to the product of the charge on the particle and the potential gradient. This force will be opposed by:

(*a*) The hydrodynamical frictional forces, which are assumed to be those given by the Stokes' theory.

(*b*) The electrophoretic resistance. When a charged particle is moving in an electric field, the oppositely charged ions around it move in the opposite direction, imparting a velocity in this direction to the water molecules. This is in consequence of the assumption that the water is at rest on the surface of these ions. This water stream, which is moving in the opposite direction to that of the charged particle, acts as a further hydrodynamical brake on it.

(*c*) The relaxation of the ionic atmosphere, due to the oppositely charged ions of the atmosphere ceasing to be distributed uniformly around the particle, but forming an oppositely charged cloud behind it. This effect acts as an electrical brake on the particle by partially neutralising the external potential gradient.

Debye and Hückel arrived at the result that the mobility and electrokinetic potential of a spherical particle is given by

$$\zeta = \frac{6\pi\eta u}{D},$$

but the assumptions involved in their analysis are unfortunately very serious, namely:

(1) All the assumptions involved in the diffuse double-layer theory.

(2) The ionic atmosphere possesses a spherical symmetry which is not destroyed either by the external field or by the motion of the dispersion medium. Thus there is assumed to be no relaxation in the atmosphere, the effect (*c*) above being assumed negligible, and the particle itself is assumed to be non-polarisable, that is the distribution of charge on the particle surface is unaffected by the electrical field. This latter assumption is accurate in most cases.

(3) The validity of the Stokes' law for the motion of the fluid around each particle.

(4) The suspension is so dilute that the motion of any one particle has no effect on any other particle. This limitation can be fulfilled in most cases. But another weak point in the result is its vagueness, no certain indication being given as to what values of η, the viscosity in the double layer, and D, the dielectric constant there, should be used, whether those of the pure solvent or those of the solution or some intermediate value. Mooney (1931) reports that he has been able to improve on this analysis, in particular by removing assumption (2) above, but he has not yet given details of his analysis.

The great weakness of this theory can now be seen. Helmholtz on the one hand, and Debye and Hückel on the other each arrive at different formulae connecting mobility with electrokinetic potential. There would be no difficulty if there were other independent but accurate methods of estimating the electrokinetic potential. There are other methods, but their theoretical foundations are no firmer than the present one, and the most that can be done is to determine if they give consistent estimates. The electrokinetic potential has been determined by three different methods for proteins (Briggs, 1928; Abramson, 1931), and by two different methods for paraffin oil (Mooney, 1924) and for glass (Abramson, 1931). Using the Helmholtz formula, the first two systems have the same electrokinetic potential from all the methods, but neither the Helmholtz nor the Debye-Hückel theory can make different methods give consistent values for glass.

Turning now to the actual measurement of the mobility of a particle, which is a perfectly definite physical quantity, namely its velocity through a stationary dispersion medium under an electric potential gradient of 1 volt per cm., the problem becomes much more straightforward. Two main methods have been employed, the macroscopic or U-tube method of Burton, in which the mean value of the mobility of a large number of particles is determined, and the ultramicroscopical method, in which the mobility is determined from measurements taken on individual particles. The principal soil workers who have used the ultramicroscopical method have been Tuorila (1928*a*), Mattson (1928), and Baver (1929). In this method a small volume of the sol is placed in a cuvette, put under the ultramicroscope, and an electrical field applied in the focal plane of the microscope. The velocity of individual particles along the field is found and the mean taken. But, unless special precautions are taken, the migration velocity of the particle is not determined in this way, for the dispersion medium is not stationary in the cuvette under a potential gradient. Between the walls of the cuvette and the suspension is an electrical double layer, and this causes an endosmotic flow of the water parallel to the walls, which sets up a circulation in the cuvette. Hence, to measure the migration velocity of the particle, it must be determined either where the solution is stationary, or the endosmotic flow must be allowed for. The first method is the one usually adopted. Smoluchowski (1914) gave the details of the circulation in the cuvette for the particular case of one formed by two infinite parallel plates, and Mattson (1928) gave the corresponding details for a long cylindrical cuvette. All workers in this field, except Mattson, have used small rectangular cuvettes bounded by either four walls or three walls and a liquid-air interface. They have assumed that such a cuvette was equivalent to one of the type contemplated by Smoluchowski and ignored the endosmotic flow due to the side walls. Unpublished data by the author shows that the error involved by this neglect can be very considerable, and there is as yet no way of correcting for this, as the mathematical calculations for the circulation have not yet been carried out. Mattson alone used a cylindrical cuvette, which is the only type for which the requisite calculations have been made. Kruyt and van der Willigen (1928*a*) pointed out one of the great disadvantages of this method, namely the great difficulty of controlling the temperature of the sol. For quantitative work this is very important, since a variation of 1° C. alters the migration velocity by 2 per cent. Another disadvantage is that only dilute solutions of electrolytes can be used in the cuvette, otherwise the electrodes become polarised very rapidly and the products of electrolysis interfere with the colloidal particles.

The other method, the macroscopic method, of measuring migration velocities consists of some such apparatus as Burton's U-tube. A complete discussion of the use of this apparatus and the suitable modifications needed to overcome the polarisation of the electrodes and other difficulties will be found in the papers by Kruyt and his co-workers (1928, 1929) and of Prideaux and Howitt (1930). The author is not aware of any quantitative work on clays in which this method has been used.

IV. GENERAL THEORY OF THE STABILITY OF DISPERSE SYSTEMS.

Factors affecting the stability of disperse systems.

A sol or suspension will be said to be stable when the dispersed particles show no tendency to coagulate. The general theory of the stability of such systems has been developed largely by Kruyt (Kruyt and Bungenberg de Jong, 1929; Kruyt and Tendeloo, 1929), and is founded on the assumptions that the stability of a sol is dependent on two factors, the electrokinetic potential and the degree of hydration of the individual particles. Thus on this theory in order to discuss the influence of any substance on the stability of a sol, it is only necessary to discuss its influence on these two factors.

The addition of a non-electrolyte to a sol can cause the following effects; it can alter the dielectric constant of the dispersion medium or the hydration of the particles, or may even be adsorbed on or react with the surface of the particles, giving a new surface having another set of properties from the original. The effect of alteration of the dielectric constant on the electrokinetic potential is complex. Most non-electrolytes reduce the dielectric constant of water considerably, and this has the following effects:

(1) If the mean thickness of the electrical double layer and the mean number of ions in it remains constant, decreasing the dielectric constant increases the electrokinetic potential.

(2) But decreasing the dielectric constant of the dispersion medium increases the forces of attraction between oppositely charged ions. Thus both the number of ions dissociated from the surface of the particle and the mean thickness of the double layer are diminished, both of these effects lowering the electrokinetic potential. This is usually somewhat offset by the lowering of the dissociation of any salts present in the solution. Martin and Gortner (1930) give a few results illustrating this

effect, but they only used water and pure organic liquids and not salt solutions.

The addition of such non-electrolytes as alcohol and acetone, which reduce the hydration of the particles, decrease the stability of the particle, since a hydrated particle has a certain measure of stability due to its hydration alone. If the hydration is due primarily to the ions in the outer layer of the double layer, as with clay particles, reducing the hydration reduces the thickness of the double layer, since the ions can now get closer to the surface, and thus reduces the electrokinetic potential of the particle. Whang (1931) gave some interesting results with quartz suspensions illustrating these results with alcohol.

The addition of an electrolyte to a sol can cause the following effects: it can alter the electrokinetic potential by altering the dielectric constant, the thickness and the mean number and types of ions in the double layer, it can alter the hydration of the particles, as for example in the "salting out" process used for the coagulation of certain lyophilic colloids, or it can alter the surface chemically, giving a new surface having a different set of properties. The ions that act as coagulators are those carrying a charge of opposite sign to that on the particle's surface. In dilute solutions, if the ions carrying a charge of the same sign do not react chemically with, nor are adsorbed by the surface, they affect the electrical properties of the particle mainly through their influence on the activities of the coagulating ions present.

The effect of electrolytes on the stability of colloidal suspensions.

On the theory just outlined, an electrolyte alters the stability of lyophobic colloidal sols and of clay suspensions largely by its effect on the electrokinetic potential of the colloid, provided that neither ionic exchange nor chemical adsorption of the ions is taking place. In this subsection three separate aspects of this problem will be discussed, namely the relative coagulating power of different electrolytes, the relation between the electrolyte concentration and its effect on the electrokinetic potential of the sol, and finally the relation between the electrokinetic potential of a sol and its stability as influenced by the presence of electrolytes.

Schulze (1882), Linder and Picton (1895), and Hardy (1900) showed that the coagulative power of a simple electrolyte depends principally on the valency of the ion carrying a charge opposite in sign to that on the colloidal particle, and increasing as the valency of this ion increases. For brevity this ion will be called the coagulating ion. The above result is the Hardy-Schulze valency rule, and can be expressed by the following approximate expression for hydrophobic colloids. If R', R'' and R''' be the reciprocals of the molar concentrations of coagulating ions having one, two and three valencies respectively which are required to coagulate the sol, then

$$R' : R'' : R''' = K : K^2 : K^3,$$

where K is a constant, usually between 35 and 40. Whetham (1899) gave some theoretical foundation for this result. This rule has been found to hold for a very large number of colloidal systems, and Freundlich and Zeh (1924) showed that it held also for complex coagulating ions as, for example, the cobaltamine and the gold and platinum cyanide ions.

Valency alone, however, does not entirely account for the coagulating power of ions. The coagulating power also depends on the atomic or molecular weight of the ion. In a given valency class the coagulating power of the coagulating ion in general increases as its atomic or molecular weight increases. This result appears to be general for cations and electronegative sols, but for anions and electropositive sols this is probably complicated by the tendency of large anions to form weak electrolytes, but there is very little experimental evidence for this case. For the simple metallic ions, as for example the alkali or alkaline earth ions, this is simply the Hoffmeister rule. The lithium ion is a weaker coagulator than the sodium, and this is weaker than the potassium ion. Wiegner (1925), in emphasising the validity of this rule for clay suspensions, stated it as the coagulating power of an ion decreases as its hydration, or effective ionic volume (the volume of the ion together with its hydration envelope), increases. But he only considered the alkali and alkaline earth ions. Freundlich and Birstein (1926), using an arsenious sulphide sol, showed the coagulating power of the ethyl ammonium series of ions was doubled for each substitution of a C_2H_5 group for a H atom in the ammonium radicle. They found a similar result using a ferric oxide sol and the sodium salts of the first six fatty acids. These experiments complicate the hydration explanation of the second rule considerably, for it is difficult to believe that these complex ions are small in comparison with the caesium ion, for example, which latter is generally assumed to be nearly dehydrated, or that the effective volume of the tetra-ethyl ammonium ion is smaller than that of the tri-ethyl ammonium ion. Yet hydration seems to be concerned with the simple metallic ions, for all the alkali ions are nearly equal if the colloid is markedly hydrophilic. Further, Dorfmann (1930) showed that the differences between the coagulating powers of the alkali ions decreases as alcohol is added to the solution to dehydrate them.

A third factor on which the coagulating power of the coagulating ion depends is its activity in the solution. When a coagulating ion is added to a colloidal system, an ion carrying a charge of opposite sign to it must be added also. Two new words can conveniently be introduced here. The ion carrying a charge of the same sign as that carried by the colloidal particle will be called the "homoid" ion, while that carrying a charge of the opposite sign will be called the "heteroid" ion. These two words were very kindly suggested by Prof. D. S. Robertson in place of the two German words "Nebenion" and "Gegenion" respectively, and since there exist no similar English words in current use, it is felt permissible to introduce these two new ones. Linder and Picton (1895) and Hardy (1900) showed that, for the systems they were studying, coagulation set in for a given coagulating ion but for different homoid ions when the conductivity of the solution reached a definite value independent of the homoid ion used. In general, it appears that, provided the homoid ion is not adsorbed by the colloidal particles, its influence on the concentration of the coagulating ion required to coagulate a given sol is mainly through its influence on the activity of the coagulating ion. It is not impossible that for simple systems a given coagulating ion coagulates a given sol when its activity has reached a given value, but apart from the early experiments mentioned above, there is not very much definite evidence on this point. The two rules governing the homoid-ion action are firstly the higher the valency of the ion the larger is the concentration of the coagulating ion needed to coagulate a given sol. Thus sodium chloride is a better coagulator of an electronegative sol than is sodium sulphate, and this is a better coagulator than is sodium citrate. And secondly, the weaker the electrolyte formed by the two ions, that is the less it is dissociated, the greater is the concentration of the electrolyte needed. To coagulate an electronegative sol, for example, a larger concentration of acetic acid is needed than of hydrochloric acid. Dorfman and Ščerbačewa (1930) and Pauli and Wittenberger (1930) give results illustrating the effect of the homoid ion on both electropositive and electronegative sols.

The effect of electrolytes on the electrokinetic potential was first systematically studied by Burton (1909), who showed that the power of an electrolyte to depress the electrokinetic potential of a sol depended primarily on the valency of the ion carrying a charge of opposite sign to that carried by the colloidal particles. This is parallel to the Hardy-

Schulze rule for the coagulating power of electrolytes. This result has been carefully verified later by Freundlich and Zeh (1924) and by Kruyt and van der Willigen (1928 *b*) among others. The latter two authors and Tuorila (1928 *b*) also showed the second rule could be taken over as that in a given valency class the power of a heteroid ion, that is one carrying a charge of opposite sign to that carried by the colloidal particles, to depress the electrokinetic potential of a colloid, increases as the atomic or molecular weight of the ion increases. And what little evidence there is, suggests that the higher the valency of the homoid ion, the higher the concentration of the heteroid ion must be to produce a given lowering of the electrokinetic potential. One important point in this theory is that Müller (1928*a*) succeeded in evaluating the relationship between the electrokinetic potential of a suspended particle and the concentration of the added electrolyte on the basis of the Debye-Hückel theory of the diffuse double layer. This relation contains only one arbitrary constant, namely the radius of the colloidal particles, which is not always known. He applied this to Freundlich and Zeh's (1924) data, and showed that the data gave a consistent value for the radius of the particles, and further that using this value his calculated curves agreed remarkably well with the experimental ones. This agreement is extremely gratifying, as it seems to indicate that the electrokinetic potential, as computed from mobility data, really has the physical significance which is attached to it. One other important result that emerged from Müller's calculations was that the smaller the radius of the particle, the higher must be the concentration of a given electrolyte to reduce its electrokinetic potential by a given amount, but that in the absence of electrolytes, the electrokinetic potential of a spherical particle is independent of its size. This result has not yet been verified, though Tuorila (1926) found some indirect evidence for it in the case of gold sols.

It is now apparent that there is a close relationship between the power of an electrolyte to coagulate a sol and to depress its electrokinetic potential. Hardy (1900) suggested that as soon as a hydrophobic sol reaches its isoelectric point, that is when its electrokinetic potential is zero, it coagulates. Powis (1914, 1916) introduced the idea of the critical potential of a sol, namely that a sol is stable until its electrokinetic potential falls below a certain critical value, when the sol begins to coagulate. He and later Bikerman (1925), Kruyt and van der Willigen (1927), and Briggs (1930) showed that for a range of sols and dispersion media a fairly rapid coagulation sets in once the electrokinetic potential has fallen below a certain value characteristic of each sol but independent of the dispersion medium or coagulating ion used. A few exceptions occur, notably when the alkali chlorides are used as coagulators of electronegative colloids, for the sol usually coagulates before the electrokinetic potential has sunk to its critical value. Kruyt, Roodvoets and van der Willigen (1926) suggested this was due to a mistake in the calculation of the electrokinetic potential, for this irregularity only seems to occur when weak coagulators are being used, so that the solutions are rather concentrated. The electrokinetic potential is calculated from mobility data, and these calculations are only valid so long as the solutions are dilute. This anomaly is not shown by large complex monovalent ions which are powerful coagulators.

Tuorila (1928*b*) put the whole theory on a more quantitative basis. He showed that for a paraffin sol, which follows Smoluchowski's theory for slow coagulation reasonably well, the constant ξ in the Smoluchowski formula, namely the proportion of the collisions between two particles which result in an adhesion, is related to the electrokinetic potential ζ of the particles by a relation of the form $\zeta = -a \log \xi$,[1] where a is a constant. Unfortunately, he only used the alkali chlorides as coagulators. He proceeded to examine the relation between the electrokinetic potential and the stability of clay suspensions, but here he could not use ξ as a measure of the stability, since it decreases markedly during the course of coagulation. Instead, he used the time taken between the adding of the electrolyte to the clay suspension and the appearance of flocs in the suspension. He found there was a simple relation between the electrokinetic potential and the logarithm of this time, which depended only on the valency of the coagulating ions, the monovalent ions causing flocs to appear sooner at a given electrokinetic potential than the divalent ions.

The effect of the exchangeable base on the stability of clay suspensions.

Clay suspensions are the only systems showing marked base exchange properties that have been examined in any detail, and in this section they only will be discussed. The important property of a clay suspension in a dispersion medium which is neither strongly acid nor strongly alkaline, is that the clay particles adsorb no anions, and only exchange cations quantitatively, so that the only method by which the charge on the clay particle can alter by a variation in the electrolyte constant of the dispersion medium is by an alteration in the degree of dissociation or in the activity of the adsorbed cations.

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[1] This formula obviously only holds over a part of the range, for when $\xi = 0$, *i.e.* when there is no coagulation, ζ presumably reaches some limiting value ζ_0. But over the range of ξ from 1 to 0·001 the equation is remarkably accurate, and in fact the correlation coefficient between ζ and $\log \xi$ is $-0{\cdot}95$. Tuorila in his paper gave the correlation coefficient between ζ and ξ which has the lower value of $-0{\cdot}89$.

Anderson (1929), Mattson (1929), and Baver (1929) have shown that suspensions of clays saturated with a divalent alkaline earth cation (Mg, Ca, Sr, Ba) are less stable and have a lower electrokinetic potential in the absence of electrolytes than clays saturated with a monovalent alkali cation (Li, Na, K) or with hydrogen, as is shown in the following table:

Table I. *Mean electrokinetic potential of clays relative to the calcium clay.*

Clay	Anderson (1929)	Mattson (1929)	Baver (1929)
Ca	100	100	100
Mg	99	—	110
K	134	—	164
Na	149	191	169
H	23	168	128

As this table shows, Anderson found that a hydrogen clay had a lower electrokinetic potential than the corresponding calcium clay, but this result has not been confirmed by other workers. Mattson, however, made the interesting observation that electrolytes depress the electrokinetic potential of a hydrogen clay much more rapidly than a calcium clay, and in fact it does not require a large electrolyte concentration to give the hydrogen clay a lower electrokinetic potential than the calcium clay. Considering clays saturated with an alkali cation, the more hydrated the cation, or the lower its atomic weight, the higher is the electrokinetic potential of the pure clay suspension, and the more stable it is. Mattson further showed that if the theory of the critical potential is valid for clay suspensions, then its value depends on the exchangeable ion present. Thus he found a critical potential of – 55 millivolts for a sodium clay, while that for the corresponding calcium clay was only – 22 millivolts.

These results throw much light on an apparent anomaly in the behaviour of many colloidal suspensions. In general, if an electrolyte is added to an electronegative sol its electrokinetic potential is depressed, unless the electrolyte is a sodium or potassium salt, when it often rises a little before falling. Mattson, for example, found that if potassium chloride is added to a calcium clay suspension, the electrokinetic potential first rises a little before falling, but if it is added to a sodium clay suspension, the electrokinetic potential falls continuously. This anomaly is probably due to secondary effects consequent upon base exchange taking place. When potassium chloride is added to a calcium-saturated clay suspension, a certain amount of calcium will be exchanged for potassium, giving a clay containing both adsorbed calcium and adsorbed potassium. The introduction of potassium into this system raises its electrokinetic potential, and if this rise more than counterbalances the depressing effect of the added electrolyte, the electrokinetic potential of the clay particles rises. But if the clay is a sodium-saturated clay, the exchange of potassium into the complex with sodium will depress the electrokinetic potential of the clay particles, so both effects of the added potassium chloride reduce the potential.

Anderson and Mattson (1926) showed that the higher the ratio of silica to sesquioxides in a clay, or the higher its exchange capacity, the higher is the electrokinetic potential of the clay suspension when saturated with a given cation, in the absence of added electrolyte, and the more stable is this suspension. Mattson (1929) was able to show that this effect was due to the greater hydration of a clay with a high exchange capacity. He showed that if clays were dehydrated by using 75 per cent. alcohol as the dispersion medium the concentration of added electrolyte is the same for different clays, as the following table, taken from his paper, shows.

Table II. *Concentration of electrolyte required to coagulate a clay suspension in aqueous and alcoholic solutions.*

Dispersion medium	Clay	NaCl coagulating a Na clay	KCl coagulating a K clay
Water	Sharkey	0·08	0·03
	Norfolk	0·004	0·003
75 % alcohol	Sharkey	0·001	0·0005
	Norfolk	0·001	0·0003

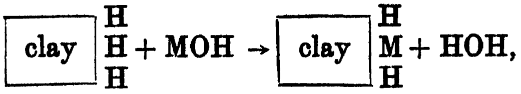
The exchange capacity for Sharkey clay is 0·796 milli-equivalent per gm. of clay, and for the Norfolk clay 0·207 milli-equivalent per gm.

The coagulation of clays in alkaline media.

A discussion on the influence of hydroxides and mixtures of hydroxides and simple salts on the stability of clay suspensions has been given recently in a monograph by Keen (1931), so only the general results of that discussion need be given here. When a hydroxide is added to a clay suspension the following three chemical reactions may take place.

1. A base exchange between the added cations and the exchangeable cations will in general occur, and this exchange can be of great importance if there is much replaceable hydrogen in the clay: for then almost quantitative replacement of the hydrogen by the cation added with the

hydroxide will take place until either all the added hydroxyl ions have been removed, or all the hydrogen replaceable under the existing conditions has been displaced. The reaction proceeds according to the following diagrammatic scheme:



$$\boxed{\text{clay}}\begin{matrix}\text{H}\\\text{H}\\\text{H}\end{matrix} + \text{MOH} \rightarrow \boxed{\text{clay}}\begin{matrix}\text{H}\\\text{M}\\\text{H}\end{matrix} + \text{HOH},$$

where MOH is any soluble metallic hydroxide.

2. If an excess of hydroxide is added, silicate and aluminate salts may be formed with the added cation. The alkali salts so formed are soluble but usually only weakly dissociated, while the alkaline earth salts are insoluble. Both of these reactions result in an alteration in the chemical properties of the surface of the particles, which may be fundamental when insoluble salts are formed on them, and in the appearance of various complex anions in the solution.

3. If other types of cations are present in the solution besides those added as hydroxides, insoluble hydroxides may be formed. Thus if sodium hydroxide is added to a clay suspension containing magnesium chloride, insoluble magnesium hydroxide will be formed. This is precipitated in the first instance either as a colloid or as a coating on the surface of the clay particles. Thus a complex heterogeneous suspension results.

Further, when interpreting the results of experiments made on the coagulation of clay suspensions in alkaline media, two points should be borne in mind, namely that a sodium clay is more stable than the corresponding hydrogen clay, and that sodium ions are weak coagulators, so that a large concentration of them are required for coagulation. If these are added as sodium hydroxide, the suspension is usually so alkaline when coagulation sets in that considerable decomposition of the clay has taken place.

Following on these lines the interpretation of most of the experimental data becomes quite straightforward. As examples, the data of Bradfield (1923, 1925, 1928), Oakley (1927), and Mattson (1929) have been discussed on these lines in detail in Keen's monograph. But there is one set of results whose interpretation is still uncertain, namely those classified in the monograph under the name of the second type of anomalous flocculation of clay suspension. A suspension will be said to flocculate when the clay settles in large loose aggregates, or flocs, and to be completely flocculated when these flocs settle, leaving a clear dispersion medium. In general, when a very dilute clay suspension coagulates it never clears completely unless centrifuged, and the complex particles are usually not visible to the naked eye. A characteristic of the coagulation of clay suspensions in alkaline media is that flocculation usually sets in, and in the phenomenon under consideration a very rapid complete flocculation may set in, which is several times more rapid than the usual rapid coagulation in a neutral medium. Thus, apparently, flocculation cannot be caused by the sticking together of uncharged particles due to their Brownian motion. The early investigators who studied this effect explicitly, as for example Comber (1920, 1921, 1922), Mattson (1922) and Hardy (1926), only studied the effect in the presence of calcium salts, but it was not until Tuorila (1928*b*) investigated the influence of other cations that a critical discussion of this effect could be undertaken. He showed that the possibility of the formation of a weakly soluble salt was an essential part of the phenomenon. This result is apparently in contradiction to some results obtained by Gedroiz (1915) and Oakley (1926). Oakley claims that a mixture of sodium chloride and sodium hydroxide flocculates a clay suspension analogously to a mixture of calcium chloride and calcium hydroxide, but his published figures, given in the following table, show that the flocculation is not more rapid than the coagulation, so the particular effect under consideration here did not occur.

Table III. *The flocculation of a purified hydrogen clay in solutions of calcium and sodium salts.*

Concentration of Ca ions in equiv. per litre	Time of flocculation in min.		
	$Ca(OH)_2$	$CaCl_2$	$2CaCl_2 + Ca(OH)_2$
0·002	28	10	32
0·003	2	7	12
0·004	1·5	6	4·5
0·010	1·5	6	1·5

Concentration of Na ions in equiv. per litre	Time of flocculation in min.		
	NaOH	NaCl	2NaCl + NaOH
0·1	32	13	32
0·2	22	14	24
0·5	20	17	17
0·9	15	18	14

Gedroiz, using an unpurified "red clay" of unstated origin, but probably containing much replaceable calcium, showed that it flocculated a little quicker in the presence of a mixture of sodium hydroxide and sodium chloride, provided there was a sufficient concentration of sodium chloride present, than in the presence of sodium chloride alone. Tuorila, apparently unaware of this experiment, made a similar one, but was unable to repeat this observation, possibly because he did not use a sufficient concentration of sodium hydroxide. Thus, while it is possible to offer several explanations of Gedroiz's result, it is considered advisable to await a fuller investigation before discussing this effect further.

Tuorila (1928*b*) showed that if sodium hydroxide were added to a clay suspension containing the chloride of a divalent metal in solution, the more insoluble the hydroxide of this metal the lower is the concentration of the chloride required to bring about this abnormally rapid flocculation. He then showed that whenever this flocculation takes place the conditions are such that if any colloidal particles of the hydroxide were formed in the suspension they would be electropositive, and therefore of opposite charge to the clay particles. If now such a hydroxide suspension is mixed with a clay suspension an extremely rapid and analogous flocculation sets in. The course of this flocculation was followed under the ultramicroscope, and it was shown that the rate of decrease of particle numbers is much greater than is given by the Smoluchowski theory for rapid coagulation of monodisperse systems of spherical particles. The quantity A/ρ, the ratio of the attractional radius of a particle to its hydrodynamical radius, was as high as 70 after coagulation had been proceeding for 90 sec., had fallen to 20 after 6 min., and after 2 hours had only fallen to 4, while for the same clay suspension coagulating in a neutral medium A/ρ was about 2. Thus a possible explanation of this phenomenon can now be given. When a clay suspension is undergoing this rapid flocculation either an electropositive colloidal precipitate of the divalent metal is formed in the suspension, so that collisions between particles in the suspension can be caused not only by their Brownian motion, but also by the attractional forces between them; or electropositive films of insoluble hydroxides may be formed on the surfaces of some of the clay particles, making them electropositive, so that once again a system containing oppositely charged particles is formed. But before this phenomenon can be considered to be understood much more experimental data, and in particular ultramicroscopical count data, will be required.

SUMMARY.

In this paper the general theory of the coagulation of dilute clay suspensions is discussed. The more important of the points considered are summarised below.

1. Two separate mechanisms causing collisions between suspended

particles are considered, namely their Brownian motion and the mass motion of one group of particles relative to another group. Following Wiegner, these two types of collision are called perikinetic and orthokinetic collisions respectively. The rate of coagulation of a suspension is then shown to depend on the rate of collisions between particles and on the probability of adhesion between them when they collide. If this probability is unity, that is if every collision between two particles results in their adhesion, the suspension is undergoing rapid coagulation. There is excellent agreement between theory and experiment for this type of coagulation whenever the mathematical equations involved can be solved. But if the probability of adhesion is less than unity, the suspension is undergoing slow coagulation, and there is as yet no theory capable of giving the rate of coagulation of such a system.

2. The theory of the electrokinetic potential of suspended particles, and the method of determining it from mobility data are discussed. It is pointed out that at the present time there is no accurate method of measuring this potential. The method used of computing it from mobility data is not entirely satisfactory, since only inexact solutions of the mathematical equations involved are available. The lack of exact methods of measuring this potential is considered to be one of the principal weaknesses of the whole theory.

3. The influence of electrolytes and non-electrolytes on the stability and on the electrokinetic potential of suspensions is discussed and the evidence in favour of the critical potential reviewed.

4. With reference to clay suspensions, the influence of the type of exchangeable ion, and of the total amount of exchangeable ions held by the clay, on the stability of the suspension are discussed, as well as the very rapid flocculation clay suspensions can undergo in alkaline media. It is shown that all these properties can be fairly satisfactorily explained by the electrical theory here developed when due account is taken of the secondary chemical reactions taking place. Finally, it must be emphasised that in this discussion only clay suspensions have been considered that are so dilute that they show no rigidity. This puts an upper limit of about 0·1 per cent. of clay on the concentration of the suspensions discussed in this paper.

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RAPID METHODS OF EXAMINING SOILS.

I. MEASUREMENTS OF ROLLING WEIGHTS.

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(With Three Text-figures.)

In an earlier paper (1) it was shown that when pastes of soil in water are investigated with the Bingham plastometer (in a suitably modified form) one of the constants which can be determined, the so-called static rigidity, is closely correlated with the draught on the plough in the part of the field from which the sample was taken. This correlation was found only for samples taken from the same field, and there were discrepancies in the case of soil taken from a part of the field which had received large quantities of farmyard manure. In order to make these comparisons, the soils were made up into pastes having the same arbitrary moisture content, a moisture content far higher than that at which the soil could be cultivated, and since different soils absorb water to different extents, it is clear that the effective moisture contents of the pastes will only be the same when soils are compared which have very similar water-absorbing powers.

The fact that dung is known to raise the water-absorbing power of a soil fully accounts for the high static rigidity observed for a dunged soil, since the soil from this part of the field was being compared at what was, in effect, a lower free-moisture content than that of the other soils.

Attempts to compare soils of *equivalent* as opposed to *identical* moisture contents in the paste form have led to the development of the flow-plasticity test (2, 3), but this does not measure the "heaviness" of a soil in any ordinary sense, and has the disadvantage that it also operates at a moisture content far higher than that possible for cultivation.

In investigating the significance of the flow-plasticity test, the plasticity of clays was studied by measuring into how thin a thread or wire the clay mass at its most plastic moisture content could be rolled. These rollings were first done by hand, but later a machine was constructed for the purpose (4). This method is not suitable for whole soils, since sandy particles upset the degree of thinness to which the thread of soil can be rolled; but it was observed that the *weight* which had to be applied to a thin cylinder of wet soil just to cause it to elongate varied in a simple way with the radius and length of the cylinder, and differed very much for different soils. The original machine was then modified somewhat to measure this weight.

In this way soils are compared at different moisture contents, but in all cases in their most plastic state, and at moistures not so very much greater than those at which they are normally cultivated. A detailed description of the technique of the test (which is extremely rapid) and of the machine used, are given at the end of this paper, but at this point it is sufficient to explain that a small shaped cylinder of wet soil is rolled out between two plates, one being of wood and the other of lightly ground glass, so that the length of the cylinder can be observed all the time, and that the rolling is carried on under an ever increasing load, until the cylinder begins to "roll out" (lengthen and thin), and that this critical weight (W) is believed to give a good measure of the "heaviness" of the soil. Smooth glass plates are not used, since with them there is a tendency for the soil cylinder to "slip" rather than to roll, resulting finally in its flattening out before lengthening. The wood and ground glass surfaces prevent this to a considerable extent (see note on p. 143 under "Technique").

On theoretical grounds, one would expect that there would be a definite stress per unit area on the surface of the cylinder at which lengthening and thinning would just start, and that for this reason the values of W measured should be directly proportional both to the radius and to the length of the cylinder of soil. The length relationship is practically inevitable, but the fact that experiment shows good proportionality between radius and W over a considerable range is important. It not only means that workers in different laboratories need make no attempt to use cylinders of soil of any predetermined arbitrary radius, but that results obtained at any known radius will be comparable when multiplied by the appropriate constant. This also shows clearly that W is a well-defined property of a particular soil.

The relationship between radius and W is shown in Fig. 1.

The point of smallest radius is queried, because at the time that the observation was made, it was realised that the smallness of the radius rendered the determination uncertain. It was not possible with the existing machine to extend the range to values of R much greater than 2 mm., since cylinders as big as this did not turn round completely in the rolling, and consequently tended to flatten.

Various methods have been suggested in the literature for studying soil heaviness by determining the stress required to deform a mass of soil, or by some similar measurement ((5, 6, 7), etc.)[1], but these suffer in general from the defect, not shared by the rolling test, that the results depend to so large an extent on the dimensions and shape of the apparatus used.

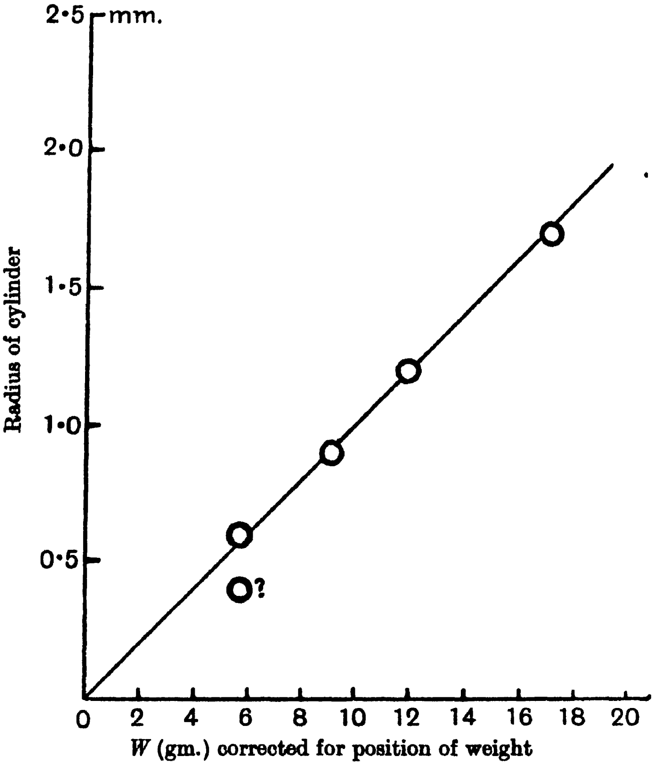

Fig. 1. Effect of cylinder radius on *W*.

W is a measure of soil heaviness in a much more fundamental sense than mechanical analysis can ever be, since the latter, even when freed from the disadvantages of arbitrary limits of particle size, can tell us only of size distribution, and it is well known that many soils differ so much in quality that two soils may have exactly the same mechanico-analytical properties and yet differ very widely in heaviness and agricultural workability.

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[1] The most satisfactory test hitherto proposed is probably that of Baver (8), who finds that the force required to pull a chisel through the soil gives a good measure of heaviness. This is related to other soil properties irrespective of the angle at which the chisel is set.

Unfortunately, data is not available for drawbar-pulls for soils in different parts of the world in such a way as to get an intensive and accurate series of comparisons. All that can be offered at the moment is a correlation of our heaviness factor with the draught on a single field (Broadbalk), the same field as that investigated in the earlier paper, a field of which we have a good intensive drawbar-pull map. Table I gives the values for *W* and drawbar-pull for a series of soils taken from Broadbalk, the drawbar-pulls being taken from the same series of measurements as those quoted in the earlier paper.

Table I. *W values for Broadbalk soils compared with drawbar-pull figures.*

Soil	18/1	16/1	14/8	14/1	7/1	10/6	16/6	5/3	18/10
Drawbar-pull	1435	1350	1230	1180	1140	1070	1065	990	980
W	17·1	14·4	12·8	10·7	9·9	9·6	9·1	9·4	9·6
Soil	5/6	5/10	2/1	7/8	2/7	2/4	7/4	7/5	
Drawbar-pull	980	954	950	938	936	898	895	877	
W	8·0	9·4	8·9	9·1	6·7	4·8	6·4	6·2	

Correlation coefficient = 0·927.

In order to show that *W* is only related to mechanical analysis provided that similar materials are compared, measurements were made on four materials whose clay contents did not differ greatly. Table II shows big differences in the values for *W* quite in accord with the general properties of this material.

Table II. *W values for four materials having high clay content.*

	W	Clay content (oven-dry)
Kaolin (*A*)	14·4	—
Kaolin (*B*)	16·6	42·7
Soudan soil	35·2	53·0
Palestine soil	49·6	53·0

In order to get some idea of the sort of range of *W* values that are obtained with different soils, values for forty different soils are shown in Table III.

(Note. After these measurements were made a slight improvement was effected in the design of the machine, so that greater accuracy could be obtained. It is probable that there is a small constant error in the values of *W* quoted in Tables I–III in the sense that they are likely to be slightly high. This is not nearly great enough in any way to affect the validity of our conclusions, or to justify a repetition of the tests, but is mentioned for the sake of completeness.)

Table III. *W values for various soils.*

Origin of soil	Identification No.	Mean *W*	Salt content	% clay oven-dry basis
Sind, India	S. 1	7·5(*f*)	22·3	15·8
,, ,,	S. 2	4·1(*f*)	4·5	10·0
,, ,,	S. 3	8·6	3·3	24·5
,, ,,	S. 4	18·0	0·25	35·5
,, ,,	S. 5	13·2	0·25	39·0
,, ,,	S. 6	17·2	17·6	17·8
,, ,,	S. 7	6·4	2·6	15·0
* ,, ,,	S. 8	17·0	2·2	36·5
* ,, ,,	S. 9	8·1	0·45	24·7
,, ,,	S. 10	5·8	0·2	25·5
,, ,,	S. 11	3·5(*f*)	0·25	18·5
,, ,,	S. 12	3·8	0·15	17·5
,, ,,	S. 13	24·5	0·25	43·7
Punjab, India	P. 28	5·7	—	16·0
,, ,,	P. 38	7·5	—	17·0
,, ,,	P. 36	4·1	—	13·5
,, ,,	P. 34	4·6(*f*)	—	17·5
,, ,,	P. 37	2·5	—	16·0
,, ,,	P. 33	5·9	—	12·0
,, ,,	P. 14	2·1(?)	—	10·5
,, ,,	P. 10	16·1	—	23·7
,, ,, Bari alkali		11·2(*f*)	2·1	16·0
Garforth, Yorks		20·4	—	22·0
†Craibstone, Scotland		5·9(?)	—	1·5
Gold Coast	G.C. 26	17·1	—	32·0
,, ,,	G.C. 28	13·9	—	38·0
,, ,,	G.C. 32	15·5	—	36·0
roadbalk, Rothamsted	Bk. 18/1	16·6	—	29·0
,, ,,	Bk. 5/3	5·9	—	16·0
,, ,,	Bk. 18/10	6·9	—	18·0
,, ,,	Bk. 14/8	12·4	—	26·0
,, ,,	Bk. 2/6	8·6	—	22·0
,, ,,	Bk. 16/4	9·1	—	28·0
Natal, Africa	N. 15	7·5	—	15·0
,, ,,	N. 18	1·6(?)	—	18·0
,, ,,	N. 46	8·0	—	29·0
,, ,,	N. 57	12·9	—	50·0
,, ,,	N. 58	4·3	—	47·0
,, ,,	N. 60	20·4	—	32·0
,, ,,	N. 49	11·8	—	30·0

* In comparing values with those in Table IV see note on p. 138.

† This sample contains much fine gravel and coarse sand. All this had to be removed before a coherent cylinder could be made. The result quoted is not therefore really typical of the whole soil.

(*f*) See note on p. 143.

The following points are worthy of notice:

1. *The Sind soils.* Several of the Sind soils contain a high percentage of soluble salts which consist mostly of Na_2SO_4 and NaCl.

In general, there is a good correlation between *W* and clay content within the group, but the correlation is disturbed by the presence of much salt, and *W* tends to be higher for surface soils (Nos. 1, 4, 6 and 9).

A subsidiary experiment was done on the effect of adding NaCl to Nos. 8 and 9, with the results shown in Table IV.

Table IV. *Values of W for Sind soils (Nos.* 8 *and* 9) *made up in solutions of NaCl of various strengths.*

Normality NaCl	0	0·1	0·2	0·5	1·0	2·0
W soil (8)	15·4	15·3	18·5	21·4	14·5	11·8
W soil (9)	8·1	9·4	13·2	15·0	7·0	5·7

It is clear that the addition of small quantities of NaCl increases the value of *W*, whereas larger quantities decrease the value.

2. *The Natal soils.* Most of these soils when first mixed up with water have a "sandy" feel, and it is at first very difficult to form a coherent cylinder from them, also when such a cylinder has been formed, it crumbles or even "rolls out" under a very small weight. In the majority of cases, however, more persistent working in the hands reduces the soils to a smooth clayey consistency producing good cylinders which require considerably greater weights to cause them to elongate. There is in reality a very tenacious crumb structure, and it is perhaps unfortunate that it is not possible to get a reliable measure of *W* both before and after it has been destroyed, since it is questionable as to how far the thoroughly kneaded soil mass reflects the properties of the original soil in the field. The Soudan soil quoted in Table II showed the same phenomenon, though to a less extent.

3. *The Punjab soils.* Some of these are near the limit of lightness for which the test is applicable. An attempt to use the rolling test for soil containing high percentages of sand (soils from the Woburn Experimental Farm) showed that it was not suitable for such soils, since it is not possible to obtain a coherent cylinder. This is not considered to be a serious limitation to the use of the method, since on very sandy soils, heaviness is not a predominant agricultural factor.

THE PACHIMETER.

It has been decided to call the machine which is used for measuring the heaviness of a soil a *Pachimeter.*

It is probable that that property of a soil which the Greeks designated by "παχύς" corresponded closely with our term "heavy" (compare Xen. *Oeconomicus*, XVII, 8). In the case of pasty materials, παχύς referred to a high consistency, hence the word "Pachoidal" suggested in an earlier paper (9). The two properties have much in common. Photographs of the pachimeter are shown in Fig. 2.

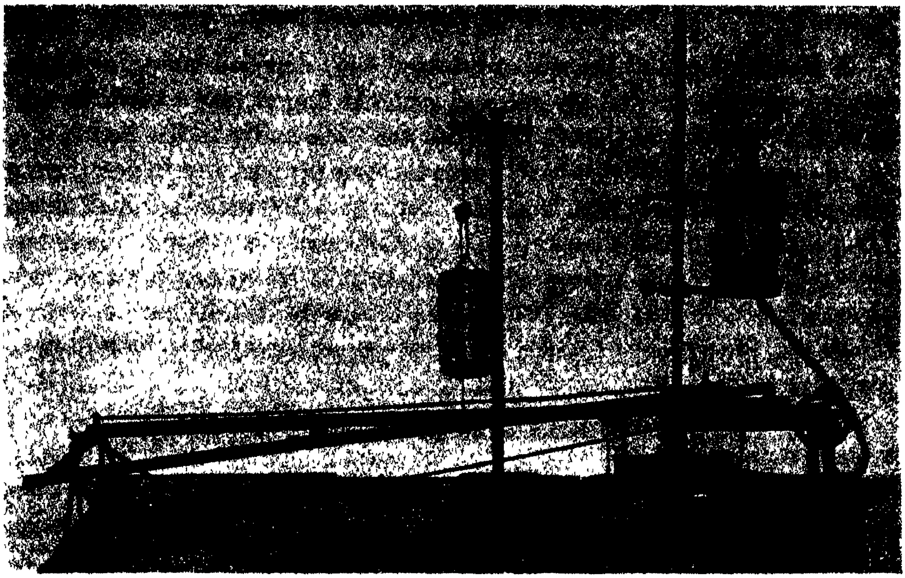

Fig. 2 *a*.

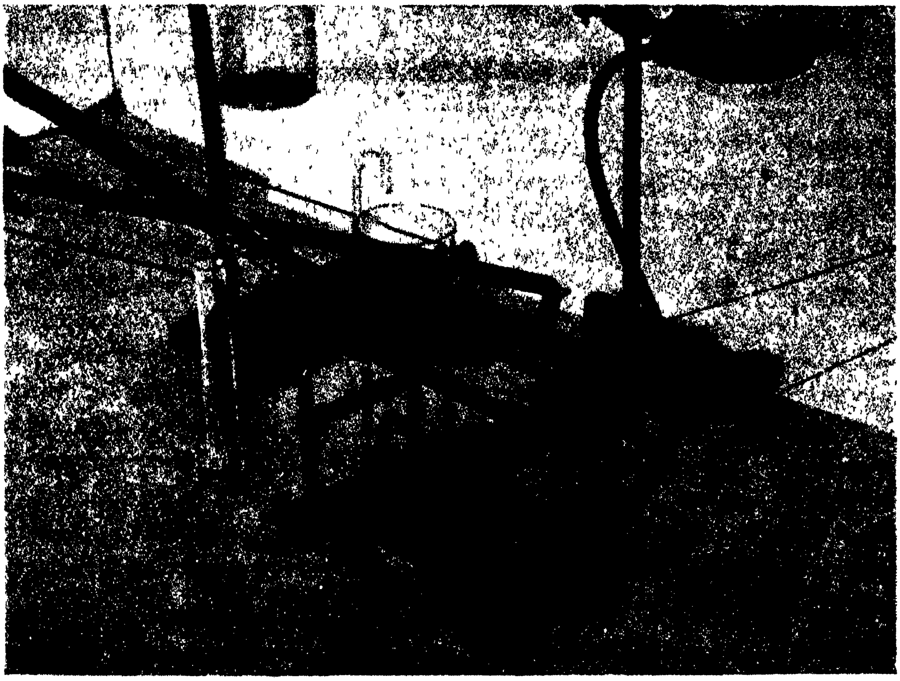

Fig. 2 *b*.

TECHNIQUE OF THE ROLLING TEST.

A few grammes of the soil are wetted with distilled water, and then worked up with a nickel spatula and with the fingers into a thick dough[1] of such a consistency that it just does not stick badly to the hands.

In most cases grits can be discarded in this process, but in very gritty soils the sample must first be passed through a 100 mesh per inch sieve. The pellet is then rolled roughly into a cylinder of about 2 mm. diameter, and is placed on the rolling board, where it is rolled out between two pieces of wood fitted with stops so as to ensure its ultimate standard thickness. Measurement under the microscope shows this to be 1·78 mm. diameter for the work described in this paper.

A piece of the cylinder is cut off by two razor blades fastened parallel to each other and 1 cm. apart: the ends of the cylinder of soil are very gently smoothed with the finger, and it is placed on the wooden reciprocating plate of the machine. It is placed approximately in the centre, with its axis at right angles to the line of reciprocation.

The motor is started, and the beaker placed carefully in position, thus allowing the upper plate just to rest on the cylinder. Water is run slowly into the beaker, and the cylinder of soil should at once start to roll. The correct moisture content for the test is the highest at which it will roll without sticking to, or dirtying the plates. Should it stick to, or dirty the plates, it must be discarded. Since if the sample is too dry it is likely to crumble before rolling out, it is not usually difficult to be certain that the correct moisture is being used.

Since the cylinder does not remain quite stationary with respect to either plate, it is not possible to use a fixed scale to observe changes in length, so that a movable millimetre scale is held in one hand in such a way as to observe at once the point at which the length of the cylinder starts to increase. At this point, the flow of water is immediately stopped, the water is poured into a measuring cylinder, and its volume recorded. The motor is stopped, the plates wiped clean, and the test repeated. It is sometimes possible to make a repeat by cutting another centimetre length off the originally rolled cylinder, but if this is done, great care must be taken that there has been no serious drying out in the interval. Should duplicates not agree within a gramme or so, a third and entirely independent test should be carried out.

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[1] It is important to make sure that the soil mass is so well worked that the crumb structure is entirely destroyed. Failure to do this is liable to cause grave errors with certain soils.

Since the weight required just to cause the cylinder to lengthen is proportional to its radius, the weight corresponding to that required for a cylinder of 1 mm. radius is obtained by multiplying the recorded weight by 1·12. The water is, however, not applied directly above the cylinder of soil, causing the necessity of a correction for difference in moment. This, for the machine used to obtain the data quoted in this paper, amounts to 0·955. Hence the product *1·07* is the factor by which all recorded weights are multiplied to get the standard rolling weight.

Tendency to "flatten." It is found especially with alkaline and saline soils that certain samples, even at the optimum moisture content, show a tendency to develop an eccentricity while rolling at a weight rather less than that required to cause an increase in length. In a few cases this is a serious inhibition to accuracy of measurement, but it is generally possible, if the rolling is stopped immediately the flattening starts, to give the sample a touch with the finger which will re-establish its cylindrical shape sufficiently to ensure its not flattening again until the critical stress has been reached.

This manipulation may cause a slight increase in length (perhaps about 0·5 mm.) which must be duly noted, and not confused with the later increase under the application of further weight.

Since this involves the possibility of a certain small error in determining W owing to the fact that length and radius no longer have quite their standard values, in the case of any test where this procedure was adopted, the W value is marked (f).

Time of the test. It is found that, in general, with normal soils, and after a little practice, the preparation of the sample, the carrying out of the test in duplicate, or in triplicate if required, and the recording of the results, takes about 7 min. It is thus possible to test about nine soils per hour[1].

Speed of reciprocation. It has been found that the critical stress varies only slightly with changes in rate of reciprocation, but it is convenient to use a fairly constant rate. If the rate is too great, the sample is apt to be jarred, and to roll crooked—if too small, there is a greater danger of flattening. A rate of about one reciprocation in 2 sec. is found convenient (thirty reciprocations per min.).

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[1] If an assistant is available to prepare the cylinders, it is possible to work at a rate of about thirteen to fourteen samples per hour.

SUMMARY.

1. A method for measuring the heaviness of a sample of soil is described which has the following advantages:

(i) The test can be completed in about 7 min.; no initial drying, sieving or weighing is involved.

(ii) The apparatus is simple to construct and comparatively easy to work. The resultant figures are fundamental in nature, and not dependent on the dimensions of the apparatus used.

(iii) The property measured is the actual weight required to deform the soil, and is thus more fundamentally linked with heaviness than with such a property as the degree of subdivision as determined by mechanical analysis.

(iv) The test is done at a moisture content close to that obtaining under field conditions.

2. The W values obtained are closely related to drawbar-pull figures for a single field, and to clay content for a single group of soils, but soils of different types show widely different W values for a given clay content. W is considerably affected by the presence of salts in the soil.

3. The method is not suitable for the study of sandy soils, but this is not a serious disadvantage, since in sandy soils, heaviness is not a predominant agricultural factor.

4. The rapidity of the method should make possible an intensive survey of the soil within a given area on a scale hitherto impracticable.

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THE PACHIMETER AS AN INSTRUMENT FOR TESTING MATERIALS, WITH SPECIAL REFERENCE TO CLAYS, SOILS, AND FLOURS

G. W. Scott Blair and R. K. Schofield

In an earlier paper[1] concerning the deforming of plastic materials the distinction has been emphasized between (*a*) the extent to which a material can be deformed without rupture, and (*b*) the stress required to cause this deformation to start to take place. The former (*a*) (which formed the main consideration of the earlier paper) is the plasticity, and the latter (*b*) is in the nature of a yield-value or shearing strength.

In a two-phase system such as soil-water, clay-water, flour-water, paint-oil, etc., one is faced with two troublesome questions in interpreting measurements either of (*a*) or (*b*): first, is the property a variable depending on the ratio of the disperse to the continuous phase, and, if so, at what ratio must it be determined; and second, is it possible to get figures independent of the dimensions and conditions of the apparatus used for the determination? Ways of overcoming these difficulties in the case of plasticity have been discussed in the earlier paper, both the flow-plasticity test, and the less quantitative but more direct wire test giving figures independent of apparatus design, and not dominated by the moisture contents.

In view of the importance of (*b*), it was thought advisable to investigate methods for measuring this property in an analogous way. The determination of the yield-value, or the shearing strength of a paste of the material is a simple matter, but this varies widely with phase-ratio, and to compare different systems at the same phase-ratio is quite misleading for various reasons, foremost of which is the complication caused by different degrees of solvation. Since the effective volumes, calculated from the viscosities of dilute suspensions by means of Einstein's equation, indicate a different degree of solvation, so in the case of pastes it is natural to expect that unequal amounts of water will have to be added to different materials in order to bring them to a comparable condition.

The investigation of systems having an optimum phase-ratio for mouldability is by no means new, but, in order to make such a method dependable, steps must be taken to ensure that the attaining of the optimum condition is sure, and that any deviation from it is readily detected.

An apparatus has recently been made and a technique devised whereby the shearing strength, as defined above, can be rapidly measured with fair accuracy. This apparatus, which is called the pachimeter, was designed for the study of soils and clays, and has been described in the *Journal of Agricultural Science*,[2] but, since the measurement is believed to be of importance in other industries and for other materials, it is felt that a further brief description in a journal read by Rheologists working in all fields would be advantageous. It is believed that little or no alteration in the machine need be made to render it available for use for the study of many other systems.

General Description

The shearing strength, or heaviness as it is called in the case of soil, is measured by the stress required to cause a flow, and it is believed that the simplest kind of flow to study is that produced when a cylinder of the material is caused to roll between two plates reciprocating* with respect to one another, the upper plate being subjected to an ever increasing load, until the cylinder elongates and thins. The load required just to start the elongating flow is, for a given material, proportional directly to the radius and length of the cylinder within reasonable limits of these values (see below), so that the critical stress for a cylinder of standard size is easily calculated. No special dimensions for the cylinder need be used for the test, so long as these dimensions are known. In the case of soil–water and clay–water systems the material is worked as wet as possible subject to the cylinder's not sticking to the plates, and, in most cases, an inadequate amount of water results in a crumbling of the cylinder before it flows, so that there is little difficulty in fixing the optimum condition. This is, however, a point which would have to be studied separately for each two-phase system under consideration.

The method has the advantages that (1) measurements can be made on very small samples of material, a gram or so being generally adequate,** (2) very little pretreatment of the sample is required—there is no weighing, (3) the test is extremely rapid for soils or clays; the complete preparation of the sample from the crude state, the taking of the measurement in duplicate, and the calculating of the stress, takes normally about six minutes—with other more homogeneous materials it could doubtless be done even more quickly. The apparatus is shown in Figure 1.

Certain minor difficulties of technique, which had to be overcome in the case of soil work, are described in the earlier paper[2] and need not be repeated here. The technique must be modified slightly depending on the material used. The material must, however, in all cases be thoroughly worked first in the hands, otherwise the resultant stress will not be a reproducible quantity. It is also clear that any large grits, or similar ingredients, must first be removed from the material. If there are many

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* The results do not vary much with small variations in the rate of reciprocation. A rate of about 30 reciprocations per minute is found to be convenient in the case of moist clay and soil masses.

** For some materials the use of such small samples may be a disadvantage. In such cases there is no reason why the whole test should not be carried out on an appreciably larger scale.

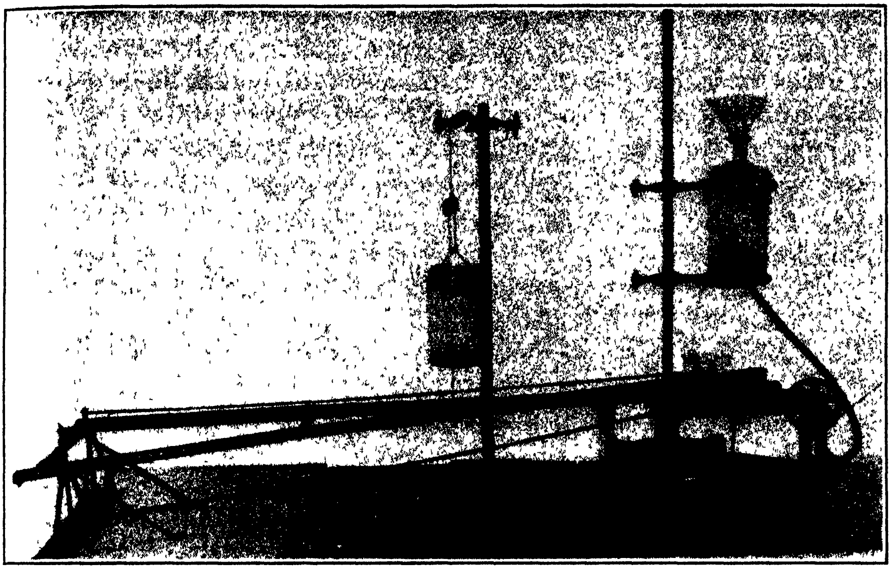

FIGURE 1A

FIGURE 1B

of these, sieving may be desirable, but in general such small samples are used for the test that grits can be removed individually with a small spatula as the sample is made up. In the apparatus used in this laboratory, a small lump of the plastic mass is rolled out between two wooden plates to a constant diameter (1.78 mm) and a cylinder 1 cm long is cut off and placed on the lower plate of the machine. Plain glass plates do not give a good grip. The upper plate is made of lightly ground glass, and the lower of wood or brass. Brass has the advantage that it can be filed to fit very truly between the guiding blocks, and it is essential that the reciprocating motion should be as even as possible. The load is applied by running water into the beaker shown in the figure, the supply of water being turned off as soon as the test cylinder starts to increase in length.

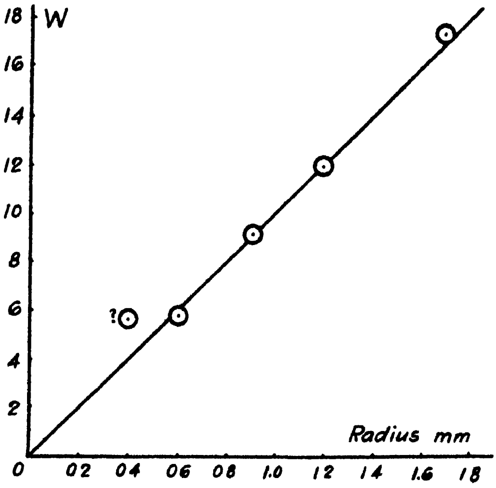

Figure 2

The length is observed by means of a small millimeter scale held in the hand. The amount of water required to start the flow resulting in lengthening has to be multiplied by a factor in order to evaluate the critical stress (*W*) for a cylinder of 1 mm radius and 1 cm length (the standard cylinder), and also a small correction should be made to allow for the fact that the load is not applied immediately above the test cylinder. In the case of the apparatus used in this laboratory, the combined factor amounts to 1.07.

The long beam of the machine is designed to ensure a minimum of friction and directional distortion in applying the load. Several small improvements have been made since the photograph shown in Figure 1 was taken: for example, the string used on the balance part of the machine has been replaced by a chain, but excepting for such minor alterations, the machine has now been used successfully in this laboratory for some months in its present form.

The Dependence of the Critical Stress on the Radius of the Cylinder

In Figure 2 is shown a curve relating the critical stress to the radius of the cylinder. Should too small a cylinder be used inaccuracies result owing to (1) large errors in determining the radius, and (2) increased danger of trouble owing to irregularities in the material. Too large a cylinder, on the other hand, will not perform a complete revolution in the course of the stroke, and so will tend to flatten out rather than to roll. It is clear that for cylinders of reasonable size, the critical stress is proportional to the radius.

Some Examples of the Results Obtained

1. *Soils and Clays.*—A large number of soils and clays have been examined, and it is found that for almost all except the light soils (where heaviness is in any case not a predominant factor) satisfactory values can be obtained representing the heaviness of the material. These range in value from about 5–50 grams as a rule, though still more extreme figures have occasionally been obtained. This wideness of scale compensates for the fact that the percentage accuracy is not very high. A discussion on the further significance of the results is given in the earlier paper, and will

TABLE I

PACHIMETRY OF SOME SOILS

Soil	No.	W. (Grams)	Soil	No.	W. (grams)
Sind, India	1	7.5	Bari Alkali, India	..	11.2
	2	4.1	Garforth (Yorks, England)	..	20.4
	3	8.6	Gold Coast	26	17.1
	4	18.0		28	13.9
	5	13.2		32	15.5
	6	17.2	Broadbalk (Rothamsted)	18/1	16.6
	7	6.4		5/3	5.9
	8	17.0		18/10	6.9
	9	8.1		14/8	12.4
	10	5.8		2/6	8.6
	11	3.5		16/4	9.1
	12	3.8	Natal (Africa)	15	7.5
	13	24.5		46	8.0
Punjab, India	28	5.7		57	12.9
	38	7.5		58	4.3
	36	4.1		60	20.4
	34	4.6		49	11.8
	37	2.5	Sudan	..	35.2
	33	5.9	Palestine	..	49.6
	10	16.1			

not be repeated here. In order to give some idea of the range of figures obtained, the values for a number of soils are given in Table I.

II Flours.—Measurements have also been made on a number of flours—figures are given in Table II. These figures show (1) that different flours show characteristic differences in shearing strength; (2) that there is evidence for a correlation between shearing strength and flour "strength." With the exception of No. 9, which comes too high in the table, the order in which our measurements set the flours does not conflict as far as is known with the order of their strengths. In the case of Nos. 2, 3, and 4, the differences in quality are just significant to the miller, No. 2 being satisfactory, No. 3 just a trifle below standard, and No. 4 sufficiently unsatisfactory to make a complaint from the baker a possibility. These, therefore, are placed in the right order, with just significant differences between each. With reference to No. 9, this shows a marked tendency to "flatten" in the pachimeter (a similar trouble has been observed in the case of some soils and clays).[2] The dough was rather sticky, probably resulting in the test being done at rather too low a moisture content. It is believed that these two factors may well account for the anomalous result. It is not claimed that the pachimeter gives an infallible measure of flour quality, but only that the results of a small number of preliminary experiments indicate that a further study of the method for the purpose of flour testing is well worth while, especially with a view to its use as a quality-control instrument in the mill.

TABLE II

PACHIMETRY OF SOME FLOURS

	Flour	W grams
*(1)	Manitoba	22.5
(2)	Commercial No. 1 (good quality)	20.7
(3)	Commercial No. 2 (satisfactory)	19.1
(4)	Commercial No. 2 (possibly doubtful)	18.2
(5)	Commercial No. 2 (doubtful)	17.2
(6)	Commercial No. 3	18.6
(7)	Commercial No. 4	16.4
(8)	Russian	15.8
†(9)	Squarehead's master	13.0
(10)	Commercial no. 5	12.1
(11)	Hard winter	11.6
(12)	Yeoman	11.6

*Rather a poor sample of Manitoba, somewhat old.

†See special comment.

Comparison in Technique between Flours, Clays, and Soils

The technique for clays and soils is essentially the same. With flour, however, the plastic mass (the dough) is more springy and elastic than is the case with clays or soils. In rolling into cylinders, the rubbing has to be done rather more carefully, and more sharply, otherwise the pellet of dough is compressed into a cylinder only as a result of an elastic deformation, and not a plastic flow at all, the resulting cylinder resuming an irregular shape as soon as the rubbing is stopped. In the machine, a faster reciprocation should be used, about 60 per minute being convenient as compared with 40 for clays and soils.

Since the consistency of doughs alters with continued working, it is best to compare doughs which have been worked for about the same time in the hands. Small differences in the time of working do not affect the results materially.

In other respects the materials behave similarly, but it is probable that in applying the machine to other systems such small differences will make their appearance.

In both soils and flours, the method has the advantage that the tests are carried out at a moisture content similar to that at which the materials are normally used. Few other consistency tests have this advantage.

Acknowledgment

The authors wish to acknowledge their indebtedness to Dr. E. A. Fisher, Director of the Research Association of British Flour Millers, for his kindness in supplying the flours used in this investigation, and for much information and advice given about them.

Figure I has been reproduced by kind permission from the *Journal of Agricultural Science.*

Summary

1. A machine (the pachimeter) is described for measuring the stress required just to start plastic flow in a deformable material.

2. This instrument has the advantage that it is easily and cheaply constructed, is rapid in its working, and normally should necessitate very little pretreatment for the sample to be tested. Very small quantities of the sample can be used.

3. A point in favor of the method is that the shearing strength is determined for a value of the phase-ratio which is not fixed arbitrarily, but is controlled by the physical properties of the material. In the case of clays, soils, and flours, the method has the additional advantage that the material is tested at a moisture content but little removed from that at which it has to be used.

4. It has been shown that the test gives important information concerning soil heaviness; that it is suitable for the study of ceramic clays; and it is suggested that it may be of value in examining flour doughs especially as a "control" instrument for the daily output of the mill. It is suggested

that the method, suitably modified, may prove of value in other industries where plastic materials are of importance.

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STUDIES ON THE CARBON AND NITROGEN CYCLES IN THE SOIL.

V. THE ORIGIN OF THE HUMIC MATTER OF THE SOIL.

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(With Three Text-figures.)

The object of this paper is to summarise and discuss the results described in the previous papers of the series (1, 2, 3, 4), with particular reference to their bearing on the origin of the humic matter of the soil and also in relation to the further development of the investigations as described in subsequent papers of the series.

The outstanding fact which emerged from the work described in Part II (2) was the marked similarity in the properties of the organic matter of soils from different plots on Barnfield and Broadbalk at Rothamsted, with regard to its behaviour on extraction with cold and hot dilute caustic soda, in spite of the different cultural and manurial treatments which the different plots have received.

The following graphs (Figs. 1 and 2), embodying the results in Table V of Part II (2) bring out this point in a striking fashion. The relation between extraction of organic carbon and time is similar in all cases. It was pointed out in the earlier paper that the differences in manurial treatment given to the plots from which these soils are derived, had brought about marked differences in the *amount* of organic carbon in the soils. It would appear, however, that these differences in treatment have had very little influence on the nature of the organic matter, so far as its solubility in alkali is any criterion.

Thus, if it is agreed that the evidence is in favour of the organic "make up" of these soils being similar, and while admitting that much more searching examination is needed before a critical comparison can be made, it is permissible to consider possible explanations of this similarity, with a view to framing a working hypothesis, the experimental testing of which may afford further information on the nature and origin of the humic matter of the soil.

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[1] **The investigations dealt with in this series of papers were carried out by or under the direction of the author, up to the time of his leaving the Rothamsted Experimental Station in 1927.**

If the treatments of the different plots, from which the soils examined were derived, are compared, it is found that the greatest difference in the nature of the plant residues added is that between plot 2 B on Broadbalk and plot 8 A on Barnfield. The organic residues received by the former were derived almost entirely from straw, added for the most part in the form of farmyard manure, but to a lesser extent as stubble and root residues from the wheat crop grown on this land. The Barnfield plot, on the other hand, received its organic matter in the form of mangold leaves, these being derived from the crop grown annually on this plot, the roots being carted off and the leaves left on the land and ploughed in. The difference between the cellulosic and fibrous material of which the straw is chiefly composed, and the more succulent leafy material from the mangolds, has not resulted in any marked difference in the alkali-solubility of the soil organic matter to which they have given rise, as shown by the following figures.

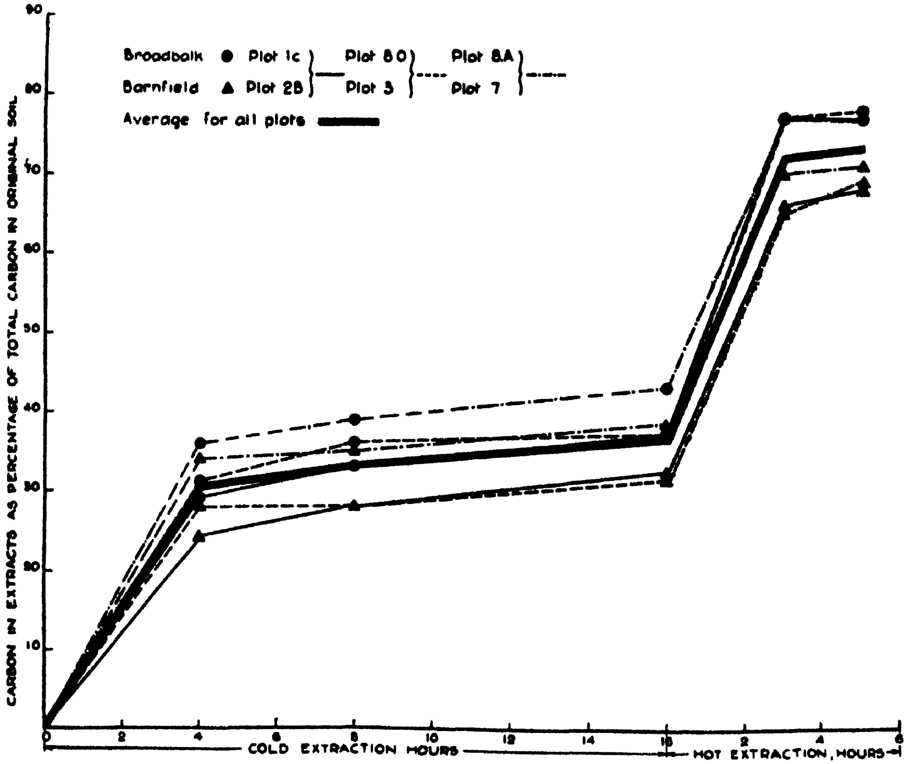

Fig. 1. Alkali extraction of carbon from surface soils.

Table I. *Alkali solubility of soil organic matter derived from straw and from mangold leaves.*

		% of total organic carbon soluble in	
Plot	Organic matter derived from	Cold dilute soda	Hot dilute soda
Broadbalk 2 B	Straw	37	77
Barnfield 8 A	Mangold leaves	38	71

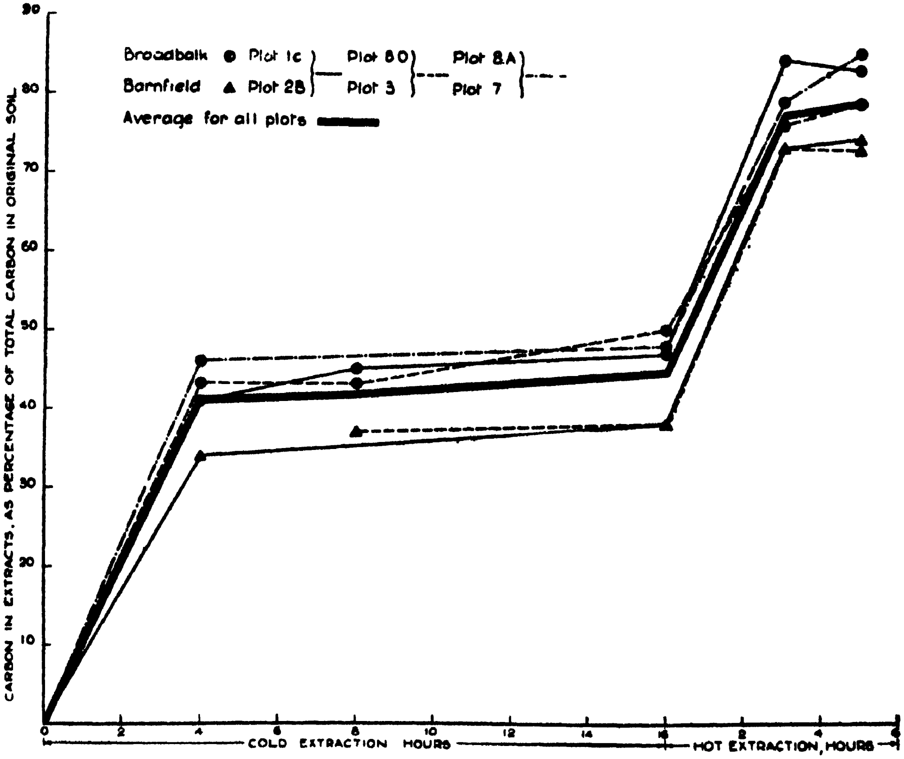

Fig. 2. Alkali extraction of carbon from subsoils.

At least two hypotheses may be advanced to account for these facts:

(1) The most widely differing organic substances in plant materials can be converted, in the soil, into the same kind of organic matter.

(2) There is one common constituent of plant materials, from which the characteristic soil organic matter is formed, the other organic substances being decomposed in the soil and not contributing directly to the soil organic matter.

Of these two hypotheses, the second appears to be inherently the more probable. Let us examine the results quoted in Part III (3) in the light of this hypothesis.

It was there shown that the formation of humic matter during the decomposition of various plant materials and of purified preparations of various plant constituents, under neutral aerobic conditions in the presence of soil organisms, was much more closely related to the changes in lignin content than to the changes in content of the other groups of plant constituents estimated. Indeed, when purified plant constituents were used, with the exception of lignin they were wholly or largely decomposed without the production of any coloured humic matter whatever.

These results thus support the hypothesis that the humic matter of the soil is produced from lignin. This hypothesis was first advanced by Fischer and Schrader (5) with special reference to the humification processes involved in the formation of lignite (brown coal). They pointed out what small chance carbohydrates had of contributing directly to the formation of dark-coloured humic matter in the soil, owing to the well-known rapidity with which they are decomposed by soil microorganisms, whereas lignin, which is relatively resistant to the action of micro-organisms, can be shown to accumulate in rotting materials, and in the presence of even weak alkaline solution it absorbs oxygen, forming a dark-coloured product resembling humic matter.

Fischer and Schrader (5) found that in the auto-oxidation of pure lignin in alkaline solution there is a splitting off of various by-products such as succinic, oxalic and isophthalic acids, so that the amount of "humic" matter formed is less than the amount of lignin from which it is derived. It is noteworthy that, in spite of the limitations applying to the estimations of humic matter and lignin in the investigations described in Part III of this series, the quantitative relation between the loss of lignin and the amount of humic matter formed, in series I and series II is closely similar to that found by Schrader (6) from the auto-oxidation of lignin. This is well shown by Fig. 3.

A conclusive proof of the origin of humic matter in the soil can be afforded only by the demonstration of the identity of the natural product with that of a product prepared under controlled conditions from a known parent substance. The investigations described in Part IV of this series (4) are a contribution to the study of the subject from this point of view. Of the many rival hypotheses regarding the parent substance of humic matter, those regarding sugars or furfural as this substance are discountenanced by the fact that the artificial humic acids prepared from sucrose and from furfural did not behave as acids on conductometric titration with ammonia, in contrast to the natural products and those prepared artificially from cellulose, hydroquinone and lignin, which showed the characteristics of true colloidal acids.

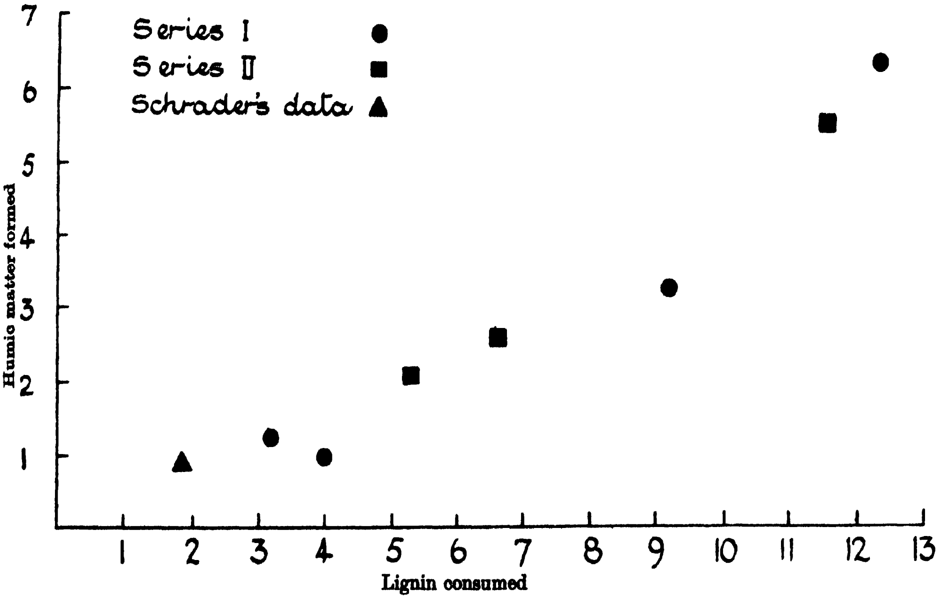

Fig. 3.

The resemblance between the natural products and those produced artificially from cellulose, hydroquinone and lignin was, however, confined to a qualitative similarity. Quantitative comparisons, whether on the basis of elementary analysis or conductometric titration, revealed important differences, not only between the natural products and the artificial ones, but also between individual substances in either class. The most striking and important difference is that in nitrogen content. The artificial products, when prepared from nitrogen-free substances with nitrogen-free reagents, were themselves nitrogen-free. The natural products, on the other hand, contained appreciable quantities of nitrogen, 5·36 per cent. in the case of the soil product, and 2·60 and 2·37 per cent. respectively for the "Adco" and peat (Dopplerite) products. The elaborate method of purification to which all these preparations were submitted, makes it unlikely that the nitrogen was present in the form of a simple admixture of nitrogenous impurities.

Oden's preparation of humic acid from peat contained only 0·7 per cent. of nitrogen, and in subsequent work on humic matter the significance of its nitrogen content has often been overlooked. Not only is the nitrogen content of humic matter a question of great practical importance in relation to soil fertility, but further progress in the elucidation of the nature and origin of humic matter necessitates investigations into the form in which the nitrogen is present in the natural product and generally into the relations between carbon and nitrogen in its formation, occurrence, extraction and fractionation. The work described in the next papers of this series is chiefly concerned with the study of this question. In the course of that work, much further information has been obtained on the nature and origin of soil humic matter and in particular on the lignin hypothesis of its origin. A considerable amount of work on this subject has also been published in recent years from other sources, but it will be more convenient to discuss this in a later paper after the results of the further work carried out in these laboratories have been stated.

At this stage it is sufficient to point out that the presence of nitrogen in soil humic matter, in a form which is not readily removed, is not incompatible with the lignin hypothesis of the origin of humic matter. The process in the soil might consist in the conversion of lignin into humic matter in the presence of nitrogenous materials which were combined in the resulting product. This possibility will be further examined, with special reference to the part played by micro-organisms in the formation of soil organic matter and in the carbon and nitrogen cycles.

SUMMARY.

The results so far recorded in this series of investigations are discussed in their bearing on the hypothesis according to which the humic matter of soil is derived from lignin.

This hypothesis is supported, but since the artificial product prepared from lignin is nitrogen-free, whereas the natural product contains nitrogen, further progress in the elucidation of the nature and origin of humic matter is dependent on a study of the part played by nitrogen in the formation of humic matter, and the form in which it occurs.

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STUDIES ON THE CARBON AND NITROGEN CYCLES IN THE SOIL.

VI. THE EXTRACTION OF THE ORGANIC NITROGEN OF THE SOIL WITH ALKALI.

BY R. P. HOBSON AND H. J. PAGE[1].
(*Rothamsted Experimental Station*[2], *Harpenden, Herts.*)

IT was pointed out in the previous paper of this series (1) that further progress in the elucidation of the origin and nature of the humic matter of the soil necessitated the study of the nitrogen content of the soil organic matter and of the relations between carbon and nitrogen in regard to the origin, nature, mode of formation and fractionation of soil humic matter. The investigations described in this paper deal with one aspect of this work.

In the second paper of this series (2) the extraction, by cold and hot dilute alkali, of the organic carbon in soil from certain plots on Broadbalk and Barnfield at Rothamsted, was investigated. With the object of tracing the relation of carbon to nitrogen in these soils, and their alkaline extracts, the same soils were examined in regard to the extraction of their organic nitrogen.

EXPERIMENTAL.

The samples of soil used were the same as those examined in the investigation described in the second paper of this series (2), and their methods of treatment and extraction were in every way identical with those described in that paper, to which reference may be made for details.

Essentially the method consists in a pre-treatment with weak hydrochloric acid to remove acid-soluble nitrogen compounds and to liberate humic matter from its combination with calcium and other metals, followed by extraction with a cold $N/2$ caustic alkaline solvent for 4, 8 or 16 hours or with the same solvent at 100° C. for 3 or 5 hours.

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[1] One of the authors (R. P. Hobson) was awarded the degree of Doctor of Philosophy by the University of London in 1926 for a thesis embodying the results described in this paper.

[2] The investigations dealt with in this series of papers were carried out by or under the direction of the senior author (H. J. Page) up to the time of his leaving the Rothamsted Experimental Station in 1927.

Nitrogen was determined in the extracts by the standard Kjeldahl method, using copper sulphate as a catalyst (no sodium sulphate was needed as the extracts already contained caustic soda). Ten cubic centimetres of A.R. sulphuric acid were added, and water removed by gentle boiling; a further 15 c.c. of sulphuric acid were then added and the digestion and distillation completed as usual.

The results obtained are stated in Tables I and II.

Table I. *Nitrogen content of Barnfield soils and their alkaline extracts. Values expressed as percentages of oven-dried soil.*

	Plot 1 C	Plot 80	Plot 8 A
Surface soils.			
Original soil	0·2595	0·0906	0·0939
Acid-treated soil	0·2558	0·0895	0·0918
Alkaline extracts:			
4 hours cold extraction	0·0805	0·0248	0·0282
8 ,, ,, ,,	0·0904	0·0267	0·0307
16 ,, ,, ,,	0·1043	0·0290	0·0346
3 ,, hot ,,	0·1968	0·0716	0·0662
5 ,, ,, ,,	0·2150	0·0730	0·0706
Subsoils.			
Original subsoil	0·1144	0·0633	—
Acid-treated subsoil	0·1126	0·0627	—
Alkaline extracts:			
4 hours cold extraction	0·0359	0·0193	—
8 ,, ,, ,,	0·0396	0·0208	—
16 ,, ,, ,,	0·0418	0·0207	—
3 ,, hot ,,	—	0·0424	—
5 ,, ,, ,,	0·0808	0·0446	—

Table II. *Nitrogen content of Broadbalk soils and their alkaline extracts. Values expressed as percentages of oven-dried soil.*

	Plot 2 B	Plot 3	Plot 7
Surface soils.			
Original soil	0·2630	0·0928	0·1153
Acid-treated soil	0·2588	0·0904	0·1123
Alkaline extracts:			
4 hours cold extraction	0·0845	0·0311	0·0374
8 ,, ,, ,,	0·1004	0·0345	0·0412
16 ,, ,, ,,	0·1051	0·0367	0·0432
3 ,, hot ,,	0·2315	0·0744	0·0921
5 ,, ,, ,,	0·2326	0·0733	0·0937
Subsoils.			
Original subsoil	0·1143	0·0726	0·0740
Acid-treated subsoil	0·1124	0·0711	0·0727
Alkaline extracts:			
4 hours cold extraction	0·0404	0·0252	0·0252
8 ,, ,, ,,	0·0442	0·0277	0·0265
16 ,, ,, ,,	0·0459	0·0302	0·0293
3 ,, hot ,,	0·0874	0·0538	0·0531
5 ,, ,, ,,	0·0910	0·0562	0·0566

Comparison of the values in the above tables is facilitated by expressing them as percentages of the total amount of nitrogen in the acid-treated soils. This has been done in Table III.

Table III. *Amounts of nitrogen in soil extracts, as percentages of the total nitrogen content of the acid treated soils before extraction.*

	Barnfield					**Broadbalk**					
	Plot 1 C		**Plot 80**		**Plot 8 A**	**Plot 2 B**		**Plot 3**		**Plot 7**	
Alkaline extracts	**Surface**	**Subsoil**	**Surface**	**Subsoil**	**Surface**	**Surface**	**Subsoil**	**Surface**	**Subsoil**	**Surface**	**Subsoil**
4 hours cold	31	32	27	31	30	32	35	33	35	33	34
8 „ „	35	36	30	33	33	38	39	37	38	36	36
16 „ „	40	38	32	33	37	40	40	40	42	38	40
3 „ hot	76	—	79	67	71	88	77	80	74	80	72
5 „ „	83	73	81	71	75	88	80	79	77	81	77

A comparison between these figures and the corresponding ones for carbon in Table V of the second paper of this series (2) reveals a striking relation between the extraction of nitrogen and carbon. A more detailed consideration of this relation, in its bearings on the form in which the nitrogen compounds exist in the soil organic matter, is deferred until a later paper, when it can be discussed along with the results of cognate investigations not yet described. For the present it is sufficient to point out that the nitrogen and carbon appear to be closely associated in such a way that under the influence of alkaline solvents they come into solution together

SUMMARY.

The alkali-extraction of the nitrogen from soils of certain plots of the classical permanent experiments on Barnfield and Broadbalk at Rothamsted, follows a closely similar course to the alkali-extraction of carbon from the same soils.

ACKNOWLEDGMENT.

The thanks of one of the authors (R. P. Hobson) are due to the Ministry of Agriculture for a Scholarship during the tenure of which this work was carried out.

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STUDIES ON THE CARBON AND NITROGEN CYCLES IN THE SOIL.

VII. THE NATURE OF THE ORGANIC NITROGEN COMPOUNDS OF THE SOIL: "HUMIC" NITROGEN.

BY R. P. HOBSON AND H. J. PAGE[1].
(*Rothamsted Experimental Station*[2], *Harpenden, Herts.*)

(With One Text-figure.)

THE investigations described in this paper were undertaken in an attempt to ascertain the forms in which organic nitrogen occurs in the soil, with special reference to the nitrogen content of humic matter. Information is required on this subject, not only for the study of the part played by the organic nitrogen compounds of the soil in plant nutrition, but also for the elucidation of the origin, nature and mode of formation of the humic matter of the soil (1).

Among earlier workers on the subject, Detmer (2) succeeded in preparing so-called humic acid containing only 0·179 per cent. of nitrogen. This was taken to prove that "pure humic acid" was nitrogen-free, but as he obtained only 1·7 gm. of the purified product from 30 gm. of crude humic acid, his results are hardly as conclusive as has been generally supposed. According to Hilgard (3), the nitrogen content of crude humic acid ranges from 1·7 to 7 per cent. in soils under humid conditions. However, certain workers have supposed that humic acid itself contains nitrogen in its structure, notably Hermann (4) and Maillard (5). Eggertz (6) found that the nitrogen could not be eliminated from humic acid by continued solution and reprecipitation; distillation in alkaline solution removed only a part of the nitrogen as ammonia. More recently Sven Odén (7) purified peat humic acid by salting out sodium humate solution with sodium chloride and obtained humic acid with only 0·7 per cent. of nitrogen.

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[1] One of the authors (R. P. Hobson) was awarded the degree of Doctor of Philosophy by the University of London in 1926 for a thesis embodying the results described in this paper.

[2] The investigations dealt with in this series of papers were carried out by or under the direction of the senior author (H. J. Page) up to the time of his leaving the Rothamsted Experimental Station.

The existence in soil or peat of protein-like bodies has been inferred from the results of various workers, including Detmer (2), Warington (8), Berthelot and André (9), Dojarenko (10), and Suzuki (11). By the action of nitrous acid, before or after hydrolysis, and in the case of Suzuki by the isolation of amino acids by Fischer's ester method, the presence in soil of bodies of a protein-like nature was established beyond doubt. The work referred to so far was essentially qualitative.

The next step was the application (mainly by American workers) of quantitative methods to the study of soil nitrogen compounds. Shorey (12), Suzuki (11), Jodidi (13), Lathrop and Brown (14), Kelley and Thompson (15), and Schmuk (16) applied the Osborne-Harris modification of the Hausmann method to the acid hydrolysate of soil. Potter and Snyder (17), Lathrop (18) and Morrow and Gortner (19) used the Van Slyke distribution method. In many cases the results obtained were of doubtful value, since, as first pointed out by Morrow and Gortner, whose paper contains an excellent account of the previous work carried out on this line, the presence of soil minerals and carbohydrates during the acid hydrolysis of a protein materially alters the nitrogen-distribution figures obtained. On account of these disturbing factors the nitrogen-distribution figures for direct soil hydrolysates have no absolute meaning; they cannot be interpreted directly and can be used only to compare different soils among themselves.

In spite of this objection, however, it is apparent, especially from the work of Potter and Snyder, Lathrop, Morrow and Gortner, and Schmuk, that the distribution of nitrogen in the organic matter of soils of different types and under different manurial treatments is similar, and not unlike that of a typical protein.

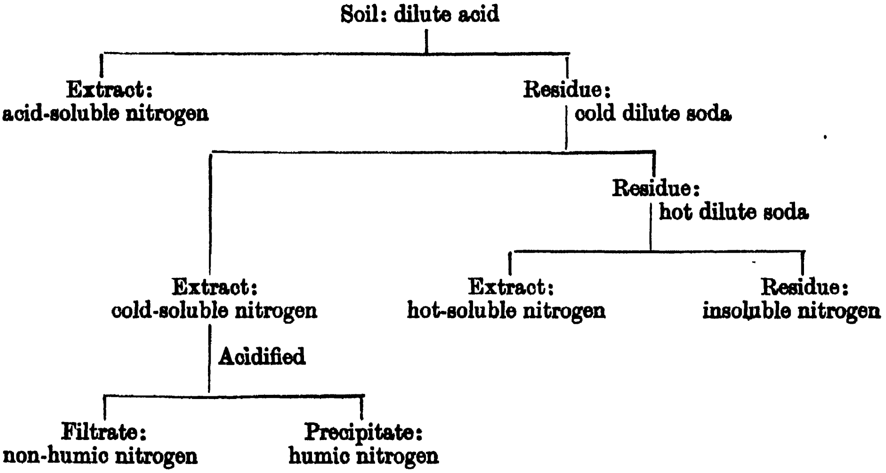
The investigations described in this paper were concerned with the nitrogen of the humic matter.

EXPERIMENTAL.

(1) *Method of extraction and nomenclature.*

A brief account of the method of extraction and fractionation is given so as to indicate the nomenclature. The soil was first extracted with a slight excess of dilute acid to remove readily soluble bases; this facilitates the later extraction of organic matter with alkali. The nitrogen extracted is termed *acid-soluble nitrogen*. The soil was then washed and extracted with cold $N/2$ caustic soda; this fraction is called *cold-soluble nitrogen*. More nitrogen comes into solution on treatment with hot $N/2$

caustic soda; this fraction is called *hot-soluble nitrogen*; the residual nitrogen is called *insoluble nitrogen.* The cold-soluble nitrogen can be fractionated by acidifying the solution; part is precipitated with the humic acid and is called *humic nitrogen*; part remains in solution and is called *non-humic nitrogen.* The scheme of fractionation and the nomenclature employed are summarised below:



(2) *Preparation of soil extracts.*

Various extracts of large quantities of soil were prepared for examination of the organic nitrogen compounds; the general method for preparing small amounts of extract already described (20) was followed as closely as possible. Phosphate and silicate which might interfere with the subsequent examination were omitted from the alkaline solvent; the soil was extracted with 2 per cent. ($N/2$) caustic soda, using 100 c.c. solvent for 10 gm. soil.

Extract I.

Surface soil from Broadbalk plot 2 B was used. Twelve separate lots of 20 gm. were acid treated and washed in the usual way. Six of these lots were extracted in the cold. After being shaken mechanically for 48 hours, and setting aside to settle, the alkaline liquid was drawn off and used to extract the remaining six lots of soil in the same way.

The alkaline extract thus prepared was used for the experiments on the removal of nitrogen from humic acid.

Extract II.

Surface soil from Barnfield plot 1 C was used. 70 gm. were acid extracted and washed in the usual way, and then extracted by shaking mechanically for 50 hours with 700 c.c. of 2 per cent. caustic soda.

The alkaline extract was filtered, acidified with hydrochloric acid and then made distinctly alkaline with caustic soda. The humic acid precipitate did not entirely redissolve on the addition of soda, but left a nearly colourless flocculent precipitate. The precipitate was allowed to settle and the clear liquid was ultra-filtered as described in an earlier paper (21). The ultra-filtrate was acidified, the clear liquid was removed, and the humic precipitate was washed once by decantation. The acid liquid removed was used for examination of the non-humic nitrogen. The precipitate was used for further experiments on purification of humic acid.

Extract III.

Surface soil from Barnfield plot 1 C was used. Two portions of 150 gm. were acid treated and washed in the usual way, and then extracted with 2 per cent. caustic soda for 2 days with occasional shaking by hand. The alkaline extract was filtered and acidified with hydrochloric acid. The supernatant liquid was drawn off, the humic precipitate filtered and washed, redissolved in alkali and reprecipitated. The reprecipitation was repeated twice more. The filtrate and washings from the first precipitation and first reprecipitation were used for examination of non-humic nitrogen. The remainder of the humic precipitate was filtered off and dried *in vacuo*; it was then used for examination of humic nitrogen.

Extract IV.

Five lots of 200 gm. of surface soil from Barnfield plot 1 C were acid extracted, washed and alkali extracted as usual; the shaking was carried out by hand, and was continued at intervals for 7 days. The acid extract and first washing were used for examination of the acid-soluble nitrogen. The alkaline extract was filtered and acidified with acetic acid. The supernatant liquid, which was not quite clear owing to incomplete coagulation of the precipitate, was used for examination of the non-humic nitrogen. The residue was used for preparation of humic acid; an equal volume of water was added and the precipitate was separated from the liquid by means of a Sharple's supercentrifuge; considerable difficulty was experienced, as the voluminous sticky precipitate rapidly clogged the machine and was not readily removed except by dissolving in alkali. The precipitate was dissolved, reprecipitated with acetic acid and again separated by centrifuging. The precipitate was twice redissolved in soda, reprecipitated with hydrochloric acid, and washed by decantation; it was then filtered off on a hardened filter paper, washed and sucked nearly dry. Washing was continued with a small amount of alcohol, when the precipitate became less sticky; it was removed and heated to 50° C. with a large volume of alcohol. The insoluble part was filtered off on the same filter, washed with hot alcohol until the washings became nearly colourless, allowed to dry in the air and then dried *in vacuo*. The alcohol extract was evaporated *in vacuo* to a small volume and the dissolved material precipitated by adding water, filtered off, washed, and dried *in vacuo*.

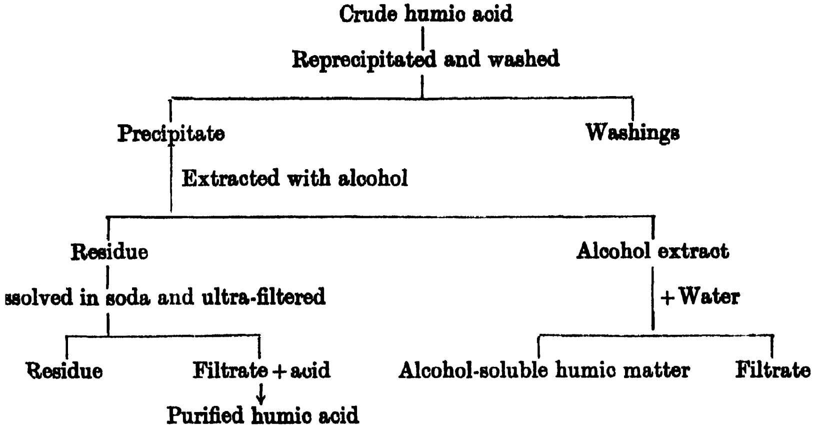
Extract V.

In this extract the fractionation of the nitrogen during the purification of the humic acid was followed quantitatively.

Five 200 gm. lots of surface soil from Barnfield plot 1 C were each shaken with 500 c.c. of dilute acid and washed in the usual way. Each lot was then extracted with 2 litres of solution consisting of 0·4 N NaOH and 0·1 N Na_2CO_3, with shaking at intervals. The nitrogen content of the supernatant liquid was determined; 40 per cent. of the total soil nitrogen was contained in the extract. 7900 c.c. of extract, equivalent to 790 gm. of soil, were taken for the preparation of the humic acid. 20 per cent. sulphuric acid was added in slight excess. After standing, the clear supernatant liquid was removed and used for the examination of the non-humic nitrogen.

The humic precipitate was filtered off, washed with a little water, dissolved in dilute caustic soda and three times reprecipitated. It was then washed by decantation, filtered, washed and left to dry. When cracking began, the precipitate, still moist, was extracted with 95 per cent. alcohol in a hardened filter paper thimble in a Soxhlet apparatus until the alcohol extracted no more colour. The residue was triturated under warm alcohol, filtered and washed with hot alcohol until the washings became nearly colourless. The alcohol-soluble humic matter was precipitated from the combined alcohol washings by adding two volumes of water made just acid with sulphuric acid. It was then washed, and dried *in vacuo*. Nitrogen was determined in the alcohol-extracted humic acid. The remainder was dissolved in alkali and ultra-filtered; the residue on the ultra-filter was washed with distilled water until no more coloured material passed through. The filtrate was acidified, and the precipitate was washed by decantation, filtered, washed, and dried *in vacuo*.

The above scheme of fractionation is summarised below:



The distribution of the nitrogen was as follows: the figures are expressed as percentages on the nitrogen in the original crude humic acid, and on the total soil nitrogen.

		Percentage of total soil N
Crude humic acid at 1st precipitation	100	19·0
Washings from reprecipitation*	14	2·7
Alcohol-soluble {Humic matter	8	2·9
Alcohol-soluble {Not precipitated by H_2O	7	
Residue from ultra-filtration*	14	2·7
Purified humic acid	57	12·7

* By difference.

The actual yields of material obtained were as follows:

Purified humic acid	4·2 gm. per kg. of soil
Alcohol-soluble humic matter	1·3 ,,

The yield of the latter is probably low; if the alcoholic extract had been evaporated to a small volume before dilution with water, a larger yield would have been obtained.

Extract VI.

600 gm. of surface soil from Barnfield plot 1 C were extracted in the usual way after preliminary acid treatment, using 6 litres of $N/2$ alkali (approximately 0·45 N NaOH + 0·05 N Na_2CO_3), and extracting for 21 hours with occasional shaking; after 3 hours' standing the alkali extract was drawn off. Four litres (equivalent to 400 gm. of soil) were

made distinctly acid with acetic acid and boiled to coagulate the humic acid. The supernatant liquid, which was not quite clear even after filtering, was removed. It was used for the preparation of the fraction of the non-humic nitrogen precipitated by basic lead acetate.

The humic acid precipitate was washed and extracted with dilute soda; the humic acid went into solution and left a white inorganic residue, showing that no protein was irreversibly coagulated by the heating with acetic acid.

(3) *Attempts to remove nitrogen from humic acid.*

(*a*) *Reprecipitation.*

The first method adopted was that of reprecipitation, by repeatedly dissolving the humic acid in alkali and reprecipitating by acid; this should gradually remove any adsorbed acid-soluble nitrogen.

Aliquot portions of Extract I (p. 499) were taken for determination of nitrogen and humic acid. The humic acid was determined gravimetrically and colorimetrically; for the gravimetric determination the humic acid was precipitated by acid, washed, dried and weighed; it was then ashed and the weight of ash deducted. The solution was also compared colorimetrically with a standard solution of Merck's Acidum Huminicum; this method does not give absolute results, but the figures may be used comparatively.

Table I. *Effect of repeated reprecipitation on nitrogen content of soil humic acid.*

	N in precipitate after operation mg.	N removed by operation mg.
1st precipitation	18·99	—
1st washing	18·49	0·50
2nd „	18·18	0·31
1st reprecipitation	17·02	1·16
2nd „	16·58	0·44
3rd „	16·17	0·41
4th „	15·85	0·32
5th „	15·56	0·29
6th „	15·29	0·27
7th „	15·12	0·17

200 c.c. portions of the extract were acidified with sulphuric acid and diluted to 250 c.c. so that the excess of acid was $N/50$, which was just sufficient to coagulate the humic acid precipitate. The supernatant liquid was removed and the precipitate was washed twice by decantation, being allowed to settle overnight after each washing. Aliquot samples of the liquid were taken at each stage for determination of

nitrogen. The humic acid precipitate was dissolved in just sufficient dilute caustic soda, and the whole of the above process repeated seven times.

The results are shown in the above table, which gives the amounts of nitrogen remaining in the precipitate after, and the amounts removed by, each operation.

The weight of crude humic acid at 1st precipitation was 363 mg.

(*b*) *Ultra-filtration.*

The humic acid precipitate remaining from one of the reprecipitation experiments was dissolved in dilute alkali and ultra-filtered; washing was continued until the liquid came through colourless. The amount of nitrogen in the filtrate and washings was determined.

The solution contained before ultra-filtration		15·2 mg. N.	
,,	after	,,	12·6 ,,

(*c*) *Dialysis.*

(i) 100 c.c. of Extract I were acidified, the precipitate washed by decantation, and dialysed in a collodion bag. Dilute caustic soda was then added and the alkaline solution dialysed for 5 days in running distilled water. The dialysed solution was then ultra-filtered and analysed.

(ii) The humic acid remaining from one of the reprecipitation experiments was dialysed until peptisation began, then dissolved in dilute caustic soda and further dialysed for 5 days. It was then analysed.

The crude humic acid after one reprecipitation contained 5·2 per cent. N.

The humic acid from (i) above contained 5·9 per cent. N.

The humic acid from (ii) above contained 5·4 per cent. N.

(*d*) *Precipitation as barium salt.*

Humic acid from Extract III (p. 500) was ultra-filtered in alkaline solution, precipitated by acid and well washed. It was then dissolved in dilute caustic soda and an excess of barium chloride was added. The precipitate was washed with dilute barium chloride solution, then freed from barium with dilute hydrochloric acid, and analysed.

Nitrogen content of humic acid before treatment		5·3 per cent.
,, ,,	after ,,	4·5 ,,

(*e*) *Salting out.*

Another portion of the same solution as that treated with barium chloride was saturated with sodium chloride. A slight precipitate was obtained, which was removed by filtration. The humic acid in the filtrate was analysed.

Nitrogen content of humic acid before treatment 5·3 per cent.
,, ,, after ,, 5·1 ,,

(f) Purification by Odén's method.

The method of purification used by Odén (7) for humic acid from peat was followed as closely as possible. Extracts of soil were made with $N/2$ and with $4\,N$ caustic soda; sodium chloride was added to the extracts to a concentration of $2\,N$; the solutions were set aside for a week. By this treatment black colloidal matter was found by Odén to coagulate and settle out. Practically no material separated out from the soil extracts; centrifuging failed to separate any suspended matter. The solutions were then nearly neutralised, concentrated on a water-bath until salt crystallised out, and filtered hot; no black colloidal material was separated by this treatment. The filtered solutions were acidified; the precipitates were dissolved in caustic soda and reprecipitated three times, filtered, washed and extracted with hot alcohol. The residues were dried *in vacuo* and analysed.

Humic acid from $N/2$ NaOH extract contained 5·4 per cent. N.
,, $4\,N$,, 3·5 ,,

(4) *Chemical nature of humic nitrogen.*

It has been shown that there is evidence that the organic nitrogen of soils may be present in the form of protein. The following experiments were designed to ascertain whether the nitrogen present in humic acid could be present in this form.

Humic acid preparations were hydrolysed with boiling hydrochloric acid and the nitrogen in the hydrolysate was examined by the Van Slyke method. The separation into basic and non-basic nitrogen, and the determination of the basic units, were, however, omitted. The nitrogen distribution studied was thus as follows:

(1) "Humin I" nitrogen[1] (nitrogen insoluble after hydrolysis).

(2) Amide nitrogen.

(3) "Humin II" nitrogen[1] (nitrogen carried down with MgO precipitate).

(4) Amino nitrogen.

(5) Non-amino nitrogen.

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[1] The term "humin" is the accepted name for the nitrogen in the insoluble coloured products formed during the hydrolysis of proteins by acids. It is here used purely in that

Humic acid was hydrolysed with boiling 21 per cent. hydrochloric acid for 40–48 hours. The solution was filtered from the insoluble residue, evaporated to dryness *in vacuo*, taken up with water and made up to 250 c.c. Aliquots were taken for determination of nitrogen and acidity. Duplicates of 100 c.c. were taken for the determination of ammonia; sufficient magnesia was added to make the solution alkaline and after adding 100 c.c. of alcohol the ammonia was distilled off *in vacuo* into acid. The acid distillate was then evaporated, made alkaline and distilled into *N*/50 acid and the ammonia determined. The residue from the distillation was filtered and the precipitate well washed; the precipitate and filter paper were taken for determination of "Humin II" nitrogen. The two duplicate filtrates were combined and evaporated *in vacuo* to a small volume. Total nitrogen and amino nitrogen were determined on aliquots; the non-amino nitrogen was obtained by difference. The amino nitrogen was determined by the Van Slyke macro-method as modified by Plimmer. The shaking was continued for 1 hour as the solution might contain diamino acids.

Samples of humic acid from Extracts IV and V (see pp. 500, 501) were examined by the above method. The humic acid from Extract IV had been reprecipitated and extracted with alcohol, but not ultrafiltered; the hydrolysis was continued for 48 hours. The humic acid from Extract V had been reprecipitated, alcohol extracted and ultrafiltered; in order to obtain better contact with the hydrochloric acid, the humic acid was dissolved in a small amount of alkali, sufficient hydrochloric acid was added to make a concentration of 21 per cent., and the mixture was boiled for 48 hours. The residue was dissolved in alkali and the process repeated. The solution rapidly coloured when heating was begun and the humic acid itself coagulated to small black lumps. Both Schmuk and Gortner mention that a violet colour formed in the condenser when a soil or soil product was hydrolysed; this was not found with purified humic acids.

The results were as follows:

Table II. *Nitrogen distribution in humic acid hydrolysates, by Van Slyke method.*

	Humic acid (Extract IV)		Humic acid (Extract V)	
	% of total N	% of soluble N	% of total N	% of soluble N
Soluble N	82·5	100	73·7	100
"Humin I" N	17·5	—	26·3	—
"Humin II" N	6·3	7·6	4·1	5·6
Amide N	15·7	19·0	15·1	20·5
Amino N	48·7	59·0	46·0	62·5
Non-amino N	12·6	15·3	11·2	15·2

Confirmation of the Van Slyke figure by formol titration.

A sample of crude humic acid from Rothamsted soil was used for this experiment. The hydrolysis and preliminary stages were carried out as usual; in this hydrolysis a violet precipitate formed on the surface of the condenser. The residue from the ammonia distillation was filtered and acidified with sulphuric acid; barium chloride solution was then added in slight excess. The precipitate of barium sulphate partially decolorised the solution and was filtered off. The solution was concentrated *in vacuo*. Amino nitrogen was determined by the methods of Van Slyke and of Sörensen.

The solution was found to contain, per 50 c.c.:

25·5 mg. amino N by Van Slyke method.
24·8 mg. amino N by Sörensen method.

(5) *Attempt to remove protein from humic acid.*

The presence of considerable amounts of amino nitrogen in the acid hydrolysates of humic acid indicated that the nitrogen was probably mainly in the form of protein-like bodies, and that possibly it might be removed by digestion with enzymes.

In a preliminary experiment, 1 gm. of crude humic acid was dissolved in alkali, precipitated with acid, filtered off and washed; the precipitate was dissolved in a known volume of standard alkali. Dilute acid of known strength and a solution of pepsin were added. The amount of pepsin added was 1 per cent. of the maximum amount of protein calculated from the total nitrogen. The amount of acid added was such that the free acid in the solution was $N/10$. The mixture, protected with toluene, was incubated for 12 days at 35° C. The mixture was then filtered and washed; the filtrate gave on neutralising a brownish flocculent precipitate, which was soluble in acids or alkalis, and thus resembled meta-protein. The biuret test was negative, but the colour of the solution was sufficient to mask this test.

The humic acid remaining on the filter was purified by reprecipitation. Its nitrogen content was 4·21 per cent. The original humic acid contained 3·28 per cent. nitrogen.

Further experiments were carried out with the humic acid preparations from Extracts IV and V (pp. 500, 501). 3 gm. of humic acid from Extract IV were digested with pepsin as above. After 5 days the solution was made just alkaline with caustic soda and sodium carbonate was added to a concentration of 0·4 per cent. A solution of trypsin (in amount 1 per cent. of the calculated protein) was added and the solution incubated at 35° C. for 2 days.

The humic acid was then purified and analysed. It contained 4·42 per cent. nitrogen; before treatment it contained 4·04 per cent. nitrogen. In order to determine whether any hydrolysis was occurring, 0·35 gm. of the same humic acid were dissolved in sodium carbonate solution, so that there was an excess of 0·4 gm. per 100 c.c. It was then incubated with trypsin for 4 days. The solution was then acidified, and the humic acid purified by reprecipitation. The filtrate and washings were analysed for nitrogen. 18 per cent. of the nitrogen was contained in the washings. The humic acid was dried and analysed; the percentage of nitrogen was 4·49 per cent.

In case the failure of the trypsin was due to the solution not being sufficiently alkaline, the experiment was repeated in a different way. 10 c.c. of $N/10$ soda were taken, excess of humic acid was added and the mixture was shaken at intervals so as to give a solution of sodium humate without any excess of soda. To the filtered solution sodium carbonate was added to a concentration of 0·4 per cent. The solution was then digested with trypsin as before. After 4 days the humic acid was precipitated; 23 per cent. of the nitrogen was found in the filtrate.

A further experiment was carried out with humic acid from Extract V. It was dissolved in sodium carbonate; the calculated excess of the latter was 0·5 per cent. Part of the solution was digested with trypsin at 35° C.; the remainder was placed in the incubator without trypsin to serve as a control. At intervals portions were removed and the amount of acid-soluble nitrogen (nitrogen in the filtrate after acidification) was determined.

The results were as follows (the acid-soluble nitrogen is expressed as percentage of the total nitrogen):

	Control %	Trypsin digest %
Zero	6	6
2 days	7	12
9 „	10	17
22 „	9	17

(6) *Comparison of natural humic acid with a synthetic mixture.*

A synthetic mixture of artificial humic acid and protein was made by adding an aqueous solution of crystalline egg albumen (prepared by Hopkin's method and dialysed until free from ammonium salts) to a solution of artificial humic acid from lignin (21) in dilute caustic soda, and then adding a very slight excess of dilute sulphuric acid. The proportions of the two substances were adjusted so that the mixture contained 5 per cent. of nitrogen. The precipitate coagulated quickly—in contrast to natural humic acid, which does not readily coagulate at very low acid concentrations. Analysis of the washed precipitate and of the combined filtrate and washings showed that over 99 per cent. of the protein nitrogen had been carried down in the precipitate. The protein alone gave no precipitate under these conditions.

When the precipitate was dissolved in caustic soda and reprecipitated by excess of acid, less than 1 per cent. of the nitrogen remained in the filtrate. When the alkaline solution of the precipitate was ultra-filtered, 77 per cent. of the nitrogen passed through the filter. When the precipitate was dissolved in caustic soda and set aside for 24 hours at room temperature—conditions similar to those obtaining during the extraction of humic acid from soils—8·2 per cent. of the nitrogen remained in solution after the mixture was precipitated by acid. When again dissolved and reprecipitated, a further 2·6 per cent. of the nitrogen remained in solution.

The mixture was dissolved in *N*/10 caustic soda and then exactly neutralised with *N*/10 sulphuric acid. Nitrogen was determined in the precipitate and the liquid. Natural humic acid was treated in the same way, with the addition of sodium chloride to a concentration of 3 per cent. to flocculate the precipitate. The proportions of the total nitrogen remaining in solution were:

From lignin-humic acid: egg albumen mixture	22 per cent.
In soil humic acid	27 „

After trypsin digestion of the mixture at 35° C. for 12 days, 74 per cent. of the nitrogen was still carried down on acidification.

EXAMINATION OF HOT-SOLUBLE NITROGEN (see p. 499).

Surface soil from Barnfield plot 1 C was extracted in the usual way with cold alkali and washed three times by decantation. The residue was extracted for 2 hours with hot 2 per cent. caustic soda.

This hot alkaline extract was cooled and the humic acid was precipitated by acidification, hydrolysed with boiling hydrochloric acid and the nitrogen distribution in the hydrolysate was determined as before (see p. 506) by the Van Slyke method. The results were as follows:

"Humin I" N	13·0 per cent.
"Humin II" N	4·9 „
Amide N	24·2 „
Amino N	47·1 „
Non-amino N	10·1 „

DISCUSSION OF RESULTS.

Purified preparations of humic acid from Rothamsted soils contained over 5 per cent. of nitrogen, thus confirming the result recorded in Part IV of this series of papers (21).

The accompanying graph (Fig. 1) shows that the amount of nitrogen removed by successive reprecipitations falls off only very slowly and that the nitrogen is associated with the humic matter in some manner not allowing of its removal by methods calculated to remove simple nitrogenous impurities which are held by purely physical means. This view is supported by the failure of the dialysis method. On the other

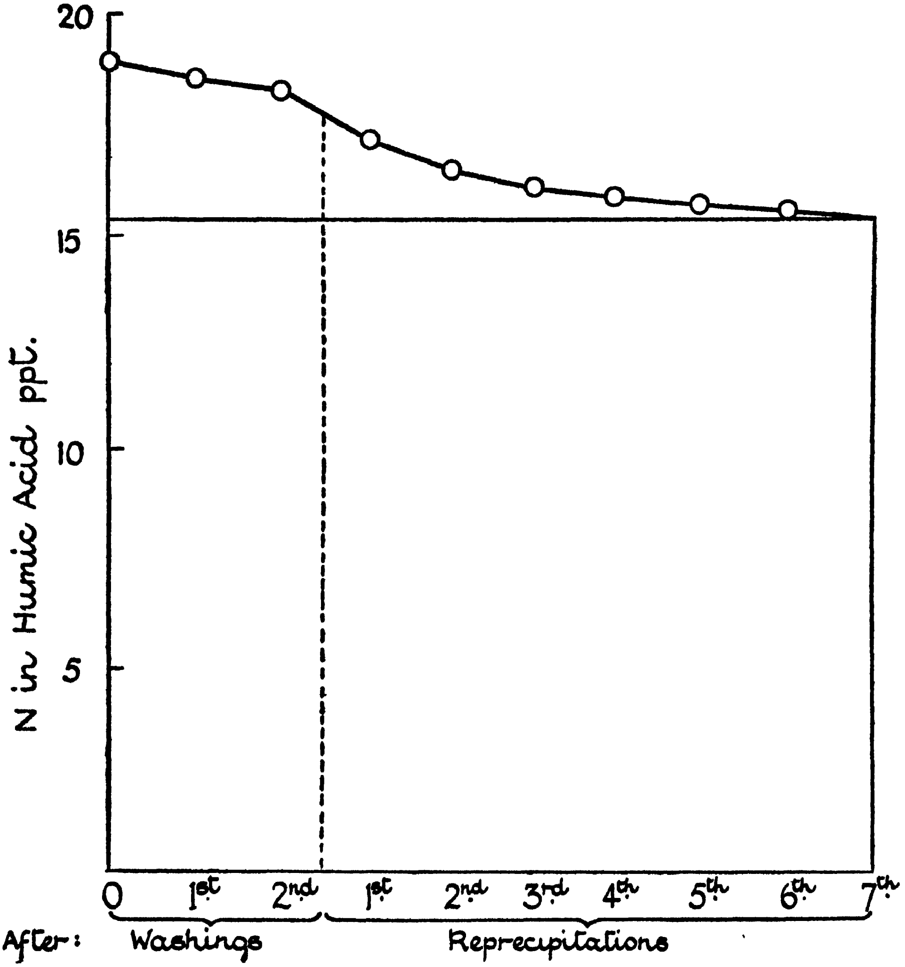

Fig. 1. Removal of N from unwashed humic acid by reprecipitations.

hand, the failure of the ultra-filtration method shows that the nitrogen must be associated with matter, the particle size of which is small enough to allow it to pass through an ultra-filter which holds back congo red; moreover, the nitrogenous part of the material is not salted out by saturated sodium chloride. In certain cases the material after treatment actually had a higher nitrogen content than before.

The above facts may be explained by supposing either that nitrogen is an integral part of the humic acid molecule or that the nitrogen is present as colloidal compounds of which the diffusibility is of the same order as that of humic acid and which are precipitated by acid or form an absorption complex with humic acid in acid solution.

The results of other workers offer strong grounds for the belief that the nitrogen of the soil organic matter is partly in the form of protein-like substances. The results described in this paper afford very strong evidence in favour of this view.

The nitrogen distribution of acid hydrolysates of humic acid was found to resemble that of a typical protein hydrolysate. Two samples of purified humic acid were examined and gave almost identical figures; the mean figures are given below and are compared with the figures from true proteins; the latter were calculated from the Van Slyke nitrogen distribution figures collected by Plimmer (22) for a number of proteins.

Table III. *Comparison of N-distribution values (Van Slyke) for soil humic acid and for typical proteins.*

	Humic acid		Mean: 9 animal proteins	Mean: 5 vegetable proteins	Limits: 14 proteins
	% of total N	% of soluble N	% of total N	% of total N	% of total N
Soluble N	78·1	100	—	—	—
"Humin I" N	21·9	—	2·2	1·3	—
"Humin II" N	5·2	6·6			
Amide N	15·4	19·7	7·4	13·9	5·2–25·5
Amino N	47·3	60·7	75·1	62·3	57·1–79·9
Non-amino N	11·9	15·2	14·9	21·7	11·5–25·8

The presence of half of the nitrogen of humic acid as amino nitrogen after hydrolysis is almost certain proof that the greater part of the nitrogen is present in some polypeptide form.

Comparison of the distribution figures with those of true proteins confirms the protein-like nature of the nitrogen of humic acid. The chief discrepancy, that of the "humin I" nitrogen, can readily be explained, and when the figures are expressed as a fraction of the soluble nitrogen a very close resemblance is found. The so-called "humin nitrogen" of a protein hydrolysis is formed by secondary reactions and is likely to be increased by the presence of other substances. It has been shown by Gortner that both carbohydrates and ferric salts increase the "humin nitrogen." It is thus to be expected that, in the hydrolysis of humic acid, which contains nitrogen in an amount corresponding to that in a mixture of one part of protein with two parts of non-nitrogenous organic matter, an unusually high proportion of the nitrogen of any protein actually present will be precipitated as insoluble "humin nitrogen."

The nitrogen distribution in the acid hydrolysate of the material soluble in hot soda was found to be very similar to that of the nitrogen of purified humic acid from the cold soda extract, as shown by the following table.

Table IV. *Comparison of the distribution values (Van Slyke) for nitrogen of humic acid from cold soda extract and hot-soluble nitrogen.*

As % of total N	N of humic acid	Hot-soluble N
"Humin I" N	21·9	13·0
"Humin II" N	5·2	4·9
Amide N	15·4	24·2
Amino N	47·3	47·1
Non-amino N	11·9	10·1

Proteins are the only class of natural compounds that yield amino acids as the principal products of hydrolysis. The fact that a large proportion of the nitrogen of soil humic acid appears as amino acids after hydrolysis therefore leads to one or other of the two conclusions: (1) that soil humic acid contains protein or protein derivatives as its principal nitrogenous constituent; or (2) that soil humic acid contains some other hitherto unknown class of compound capable of yielding amino acids on hydrolysis. Of these two alternatives the former is the more probable.

In short, the evidence so far discussed is compatible with the hypothesis that soil humic acid containing nitrogen consists of a mixture of nitrogen-free humic acid and protein. Such a mixture would not be expected to lose its nitrogen by the methods which have already been referred to in this discussion.

That a protein would remain associated with the humic acid fraction under the conditions obtaining during the extraction and purification of soil humic acid is shown by the results obtained with the mixture of egg albumen and artificial humic acid from lignin.

The behaviour of this mixture was similar to that of the natural humic acid containing nitrogen, with regard to (*a*) the retention of nitrogen on precipitation from alkaline solution with acid, (*b*) ultrafiltration of the alkaline solution, (*c*) the action of trypsin. This is shown by the following table:

Table V. *Comparison of natural soil humic acid with a mixture of lignin humic acid and egg albumen.*

Percentage of total N	Natural humic acid	Mixture of lignin humic acid and egg albumen
Retained in precipitate on addition of excess of acid	98–99	99* 97·4†
Retained in precipitate on exact neutralisation with acid	73	78
Present in ultra-filtrate of alkaline solution	83	70
Brought into solution by trypsin (excess over control)	8	23

* Without preliminary treatment with 2 per cent. NaOH.

† After preliminary treatment with 2 per cent. NaOH, and one precipitation to remove acid-soluble nitrogen produced by hydrolysis.

The failure of pepsin or trypsin to reduce the nitrogen content of soil humic acid, or to bring more than a minor part of the nitrogen into solution, is paralleled by the behaviour of the protein-lignin humic acid mixture under the action of trypsin.

In considering the difference in the amounts of nitrogen brought into solution by trypsin from the natural humic acid and the synthetic mixture, it must be remembered that protein, if present in humic acid, is probably of a different type from egg albumen, and therefore not unlikely to differ markedly from the latter in its ease of attack by an enzyme, a process in which differences in configuration or composition of the substrate may have much influence. Despite the difference in the action of trypsin in the two cases, the fact remains that only a small fraction of the nitrogen in the lignin humic acid-egg albumen mixture was rendered soluble by the action of trypsin at 35° C. for 12 days.

It is clear that the manner of association of humic acid and protein is such as largely to protect the latter from the action of proteoclastic enzymes, even in alkaline solution. That a small part of the nitrogen of soil humic acid was brought into solution by trypsin, and yet the nitrogen content of the humic acid recovered after this treatment had increased, might indicate that a part of the non-nitrogenous component of the soil humic acid was decomposed under the conditions obtaining during the incubation, thus liberating the protein originally associated with that part. A portion of the liberated protein would be converted, by the action of trypsin, into a form in which it was not carried down with the remaining humic acid when it was precipitated on acidification of the solution.

Proteins are amphoteric, and under the conditions of acidity which cause precipitation of humic acid would behave as bases. Their precipitation as part of soil humic acid could then be explained by the formation of a colloidal "salt" between the acidic, negatively charged non-nitrogenous humic colloid and the basic, positively charged protein. However, if natural humic acid containing nitrogen is made up from a non-nitrogenous humic acid and a protein, their association would appear to be a more intimate one than this. In alkaline solution, a salt-like complex of the above type would be expected to be broken up into its two component parts, and the protein would then be susceptible to extensive hydrolysis by trypsin. That it is not susceptible to such hydrolysis indicates that even in alkaline solution the protein, if present, is in some way shielded by the humic acid from the action of the enzyme.

SUMMARY.

The investigations described in this paper show that:

1. The nitrogen contained in purified preparations of humic acid obtained from Rothamsted soils cannot be eliminated by methods which would be expected to remove simple nitrogenous impurities.
2. The distribution of the nitrogen in the products of hydrolysis of these preparations of humic acid by hydrochloric acid, as determined by the Van Slyke method, is similar to that found in the hydrolysates of proteins.
3. A mixture of egg albumen and artificial humic acid from lignin resembles soil humic acid in regard to the effect of various methods of treatment on its nitrogen content. In both cases, the greater part of the nitrogen is not removed by the action of proteoclastic enzymes.

The results obtained are compatible with the hypothesis that soil humic acid containing nitrogen consists of a complex composed of non-nitrogenous humic acid and protein, and that the manner of association is more intimate than that involved in the formation of a colloidal "salt" by the mutual precipitation, in acid solution, of a negatively charged acidic colloid (humic acid) and positively charged protein on the basic side of its iso-electric point.

Further work on this subject will be described in succeeding papers in this series.

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STUDIES ON THE CARBON AND NITROGEN CYCLES IN THE SOIL.

VIII. THE NATURE OF THE ORGANIC NITROGEN COMPOUNDS OF THE SOIL: "NON-HUMIC" NITROGEN.

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(With One Text-figure.)

The previous paper in this series (1) dealt with a study of the nitrogen associated with the humic fraction of the organic matter of certain Rothamsted soils. This paper deals chiefly with an examination of the "non-humic" nitrogen of the same soils, using this term, in the sense explained in the previous paper, for the nitrogen remaining in solution when the humic matter is removed from an alkaline soil extract by precipitation with excess of acid. Earlier literature on the subject was briefly reviewed in the previous paper.

EXPERIMENTAL.

Many of the extracts used were those the preparation of which has been described in the previous paper. These extracts are referred to by number, *e.g.* Extract II, and reference should be made to that paper for details of their origin and mode of preparation. Unless otherwise stated, the soil used was Barnfield soil (Plot 1 C, surface).

(1) *Extraction of non-humic nitrogen.*

The rate of extraction of humic and non-humic nitrogen from soil was studied by extraction in the cold with $N/2$ caustic soda and $N/5$ sodium carbonate for varying lengths of time.

Portions of 20 gm. of soil were treated with dilute hydrochloric acid and then extracted in the cold with the alkaline solvent, procedure being identical with that described in an earlier paper (2). For the separation of the humic and non-humic nitrogen, from different extracts, volumes were taken such that in all cases about the same total amounts of nitrogen and organic matter were present. They were diluted and acidified with dilute sulphuric acid to give a final volume of 250 c.c. Thus the concentration of organic matter was about the same in all cases; possible errors due to adsorption were largely eliminated and comparative results were obtained. The humic precipitate was allowed to settle and nitrogen was determined in an aliquot of the clear supernatant liquid.

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[1] One of the authors (R. P. Hobson) was awarded the degree of Doctor of Philosophy by the University of London in 1926 for a thesis embodying the results described in this paper.

[2] The investigations dealt with in this series of papers were carried out by or under the direction of the senior author (H. J. Page) up to the time of his leaving the Rothamsted Experimental Station in 1927.

The results were as follows:

Table I. *Influence of time on extraction of humic and non-humic nitrogen (Barnfield surface soil, Plot 1 C).*

Solvent	Time of extraction (hours)	N in extract as % of total soil N			$\frac{\text{Non-humic N}}{\text{Total N}}$
		Humic N	Non-humic N	Total	
$N/2$ NaOH	1	7·4	12·1	19·5	0·62
	2	9·7	13·7	23·4	0·58
	4	11·3	16·0	27·3	0·59
	8	14·7	17·7	32·4	0·55
	16	17·8	19·3	37·1	0·52
$N/5$ Na_2CO_3	4	2·5	5·6	8·1	0·69
	22	4·6	7·1	11·7	0·61
	96	5·4	9·0	14·4	0·63

Sixteen hour extracts with $N/2$ caustic soda from different Rothamsted soils were compared, with the following results:

Table II. *Proportion of non-humic nitrogen in extracts from different soils.*

Soil		Non-humic N, % of total N in extract	Total N content of soil
Barnfield	1 C	52	0·260
„	8 A	80	0·094
„	8 O	81	0·091
Broadbalk	2 B	50	0·263
„	7	70	0·115
„	3	71	0·093
„	3 (subsoil)	75	0·073

(2) *Fractionation of non-humic nitrogen.*

No attempt was made to isolate individual substances from the non-humic nitrogen fraction, but various methods of fractionation were studied with a view to ascertaining the relative proportions of those groups of nitrogen compounds that can be separated by the action of different reagents. The methods of treatment used were as follows.

Alcohol treatment. An equal volume of 95 per cent. of alcohol was added to the non-humic fraction of an extract, after neutralisation and concentration to small bulk *in vacuo.*

Neutralisation precipitate. The non-humic fraction, obtained by the addition of an excess of sulphuric acid to the original $N/2$ caustic soda extracts, was exactly neutralised by adding a slight excess of caustic soda, followed by dilute acid until litmus paper just turned pink. The neutralisation precipitate (the so-called β fraction of Waksman(3)) was removed.

Phosphotungstic acid. To the filtrate from the neutralisation precipitate was added sulphuric acid to a concentration of 5 per cent., followed by phosphotungstic acid solution in slight excess. The liquid was set aside for 24 hours before removal of the precipitate.

Tannic acid. After removal of the neutralisation precipitate as above, the liquid was acidified with acetic acid and an excess of tannic acid solution was added.

Basic lead acetate. The original $N/2$ caustic soda extract was neutralised with acetic acid and a strong solution of basic lead acetate was added in slight excess.

The following table (Table III) shows the proportions of the non-humic nitrogen which were precipitated by the above treatments:

Table III. *Precipitation of non-humic nitrogen of alkaline soil extracts.*

Precipitants	Percentage of non-humic N precipitated	Remarks (see p. 516)
Alcohol	27	Extract II
„	33	„ III
Neutralisation precipitate	14	—
Phosphotungstic acid	23	—
Tannic acid	4	—
Basic lead acetate	69	Extracts IV and VI

Baryta extraction. Treatment of the original acid-treated soil with baryta solution was found to extract a much smaller percentage of the total soil nitrogen than was extracted by caustic soda (7 per cent. against 37 per cent.). Baryta was, however, found to extract the fraction not precipitable by basic lead acetate almost equally as well as caustic soda. The amounts of this fraction, after removal of ammonia, as percentages of the total soil nitrogen, were:

From caustic soda extract	3·9 per cent.
From baryta extract	3·5 „

(3) *Examination of fractions of non-humic nitrogen.*

Various qualitative tests were applied to some of the fractions obtained as above. The results are summarised in the following table (Table IV):

Table IV. *Results of qualitative tests applied to fractions of the non-humic nitrogen of soils.*

	Alcohol treatment		Basic lead acetate treatment	
Test applied	Precipitate (dissolved in water)	Filtrate	Precipitate (liberated by H_2S)*	Filtrate
Phosphotungstic acid in 5 % H_2SO_4	+	+	+(18 %)†	+(31 %)
Saturation with $(NH_4)_2SO_4$	+	.	.	.
Trichloracetic acid	+(20 %)	.	.	.
Sodium acetate and alcohol (filtrate from above)	+(15 %) (P_2O_5+)	.	+ (P_2O_5+)	.
Jaffé test for creatinine	.	−	.	−
Diacetyl test for guanidine	.	−	.	−
Biuret test	.	−‡	.	−
Murexide test	Doubtful	−	−	−
Ninhydrin test for amino acids	.	+	.	.
Van Slyke test for amino acids	.	+	.	Before acid-hydrolysis. +(30 %) After acid-hydrolysis. +(40 %)
Adamkiewicz test for indole	.	.	.	+
Fehling's test after acid-hydrolysis	Doubtful	.	+	.
Phloroglucinol test for pentosans	.	.	−	.

* Only 40 % of the nitrogen present in this precipitate could be brought into solution by the most drastic treatment with H_2S.

† The numbers in brackets, *e.g.* (20 %), indicate the percentage of the total nitrogen present in the fraction, which was separated by the treatment mentioned in column 1.

‡ Owing to the colour of the solutions, the negative result of this test cannot be accepted as conclusive.

The neutralisation precipitate, which after one solution in soda and reprecipitation by neutralisation, represented 14 per cent. of the non-humic nitrogen, contained 1·91 per cent. nitrogen and 39·3 per cent. ash, consisting mainly of silica and alumina with some phosphoric acid. Hydrolysis with boiling 21 per cent. hydrochloric acid, followed by determination of the nitrogen distribution in the hydrolysate by the Van Slyke method, was applied to the neutralisation precipitate and to the filtrate therefrom after concentration. The results were as follows (Table V).

Table V. *Nitrogen distribution (Van Slyke) in "neutralisation precipitate" and remainder of the non-humic nitrogen of soil.*

	Neutralisation precipitate %	Filtrate from neutralisation precipitate %
"Humin I" N	17·7	5·8
"Humin II" N	16·0	3·4
Amide N	24·9	27·8*
Amino N	29·5	29·9
Non-amino N	9·4	13·8

* After deduction of ammonia nitrogen present before hydrolysis.

(4) *Retention of non-humic nitrogen by the soil.*

Preliminary acid treatment of the soil extracted only a very small amount of the total soil nitrogen, and this was mainly inorganic. The original acid extract from Extract IV (see p. 516) contained about 1·5 per cent. of the total soil nitrogen. The gelatinous precipitate produced on neutralisation contained 40 per cent. of the total nitrogen in the solution. Of the remaining 60 per cent., 56 per cent. was nitrate nitrogen and 4 per cent. was ammonia nitrogen.

Extraction of Broadbalk soil (Plot 2 B) gave the following results:

0·5 *N* HCl for 2 hours extracted 1·7 per cent. of the total soil nitrogen.

0·5 *N* HCl for 1 week extracted 2·6 per cent. of the total soil nitrogen.

2 per cent. acetic acid for 1 week extracted 1·0 per cent. of the total soil nitrogen.

Since, therefore, the non-humic nitrogen which remains in solution after acidification of a dilute caustic soda extract cannot be extracted by direct acid treatment of the soil, some preliminary experiments were carried out to study the adsorption of some organic nitrogen compounds by the soil.

Soil was acid treated and washed in the usual way. It was then suspended in water and faintly acidified with acetic acid, and various simple nitrogen compounds were added. For 20 gm. of soil, the volume of liquid was 200 c.c., containing 0·75 per cent. acetic acid, and the nitrogen compounds were added to give a nitrogen concentration of approximately 1 : 100,000. Thymol was added to prevent biological action. The mixture was shaken by hand, set aside overnight and then shaken mechanically for 3 hours. The amount of nitrogen held by the soil was then determined by analysis of the clear supernatant liquid after the soil had settled. The adsorption of the non-humic nitrogen compounds obtained from a 16-hour cold $N/2$ caustic soda extract of Barnfield soil was studied in the same way, both with the acid-treated soil, and with the residual soil after an extraction for one hour with cold $N/2$ caustic soda, from which the residual soda had been removed by washing with water and dilute acetic acid. The results were as follows (Table VI):

Table VI. *Adsorption of nitrogen compounds from dilute solution by acid-treated Rothamsted soil.*

Compound tested	Percentage of added N not recovered in solution
Glycine...	12
Alanine...	11
Urea ...	0
Uric acid	9
Guanidine sulphate ...	30
Histidine hydrochloride	78
Lysine hydrochloride...	84
Arginine hydrochloride	85
Non-humic N of soil ...	57
Non-humic N of soil (adsorption by alkali-extracted soil)	64

Similar experiments in a solution faintly alkaline with magnesia showed that under such conditions lysine, histidine and arginine were adsorbed only to a much smaller extent than from acid solution, and that the greater part of the lysine adsorbed from acid solution was liberated by the action of magnesia.

DISCUSSION OF RESULTS.

The greater part of the non-humic nitrogen appears to consist of colloidal substances precipitable by basic lead acetate. The amount of nitrogen not so precipitated and not present as ammonia formed only about 20 per cent. of the non-humic nitrogen or about 4 per cent. of the total soil nitrogen. This fraction should include any amino acids, and other simple putrefaction products present in the soil. The only nitrogenous grouping, the presence of which in this fraction could be definitely established, was amino nitrogen, which was established by the Van Slyke method and by the Ninhydrin test. *Simple* polypeptides were not present to any large extent, since the filtrate from the basic lead acetate treatment showed only a small increase in amino nitrogen after acid-hydrolysis. The value of about 1 per cent. of the total soil nitrogen, for the free amino nitrogen, obtained in this work, is not very different from that of 0·5 per cent. found by Potter and Snyder(4) by the Kober method. Basic substances were present since phosphotungstic acid precipitated over one-third of the non-ammonia nitrogen not precipitable by basic lead acetate.

Potter and Snyder used trichloracetic acid to obtain a solution of the so-called "soluble non-protein nitrogen." In the present work, it was found that precipitation with trichloracetic acid removed no more nitrogen than did precipitation of the humic nitrogen with sulphuric acid. There seems little doubt, however, that the non-humic fraction includes considerable amounts of protein derivatives such as peptones, proteoses and polypeptides. The non-humic nitrogen was partially precipitated by alcohol, phosphotungstic acid, tannic acid, and basic lead acetate, all of which precipitate protein derivatives, though not exclusively.

The actual amount of nitrogen in this form can be approximately gauged by the increase in the amount of amino nitrogen on acid-hydrolysis. The free amino nitrogen formed 5 per cent. of the non-humic nitrogen; after hydrolysis 30 per cent. was present as amino nitrogen. From 60–80 per cent. of the total nitrogen of a protein is found as amino nitrogen after hydrolysis. The peptide nitrogen calculated according to this assumption would form 30–40 per cent. of the total non-humic nitrogen. Some confirmation of this is afforded by the action of phosphotungstic acid on the filtrate from the neutralisation precipitate, which precipitated 24 per cent. of the non-humic nitrogen.

Phosphotungstic acid precipitates basic compounds generally but at this high dilution it would probably precipitate only colloidal basic compounds; simple polypeptides would be precipitated only partially, or not at all. Consequently the nitrogen precipitated by phosphotungstic acid is probably entirely peptide but does not represent the whole of the peptide nitrogen. It is to be noted that Walters (8) separated a mixture of proteoses and peptones from this fraction of the soil organic matter by means of phosphotungstic acid.

The general distribution of the non-humic nitrogen is thus approximately as follows:

Peptide N...	30–40 per cent.
Ammonia N	12 ,,
Free amino N	5 ,,
Other N	40–50 ,,

The ammonia found is mainly produced by hydrolysis during the alkali extraction. As to the exact nature of the remainder of the non-humic nitrogen, very little evidence was obtained, except that it is mainly non-basic and mainly precipitated by basic lead acetate. The presence of nucleic acid (a likely soil constituent and isolated from soils by Schreiner and Shorey) was suspected but could not be established. It is not unlikely that prosthetic groups may be split off from protein during the alkali extraction to constitute a part of this fraction.

The neutralisation precipitate, which has been called the β fraction by Waksman (3), is partly inorganic, consisting largely of alumina and silica which have been dissolved from the clay during the alkali extraction. When precipitated at the neutral point, they carry down some organic matter. This organic matter thus need not differ essentially from that remaining in solution, and the results obtained by acid hydrolysis indicate that it did not actually so differ to any important extent. There would thus not appear to be any good grounds for assigning any special significance to this precipitate in relation to the composition of the soil organic matter.

The manner in which non-humic nitrogen of the soil is associated with the humic matter is a problem of considerable interest. The results reported in Part VI of this series of papers (5) indicate a close similarity in the solubility and rate of extraction of organic nitrogen by dilute alkali from different Rothamsted soils. The results reported in the present paper, however, show that the proportion of non-humic nitrogen in the organic nitrogen of the 16-hour extracts from these soils may vary between 50 and 80 per cent. These facts, together with the failure of direct acid treatment to extract from the soil the acid soluble non-humic nitrogen which accompanies the humic nitrogen in the alkali extract, indicate that the non-humic nitrogen must be associated with the humic matter in the soil in some manner which allows it to come into solution only along with and proportionately as the humic matter is dissolved.

Schreiner and Shorey (6) have already called attention to this problem, and have advanced several possible explanations. From a consideration of these, and of the results reported in this paper, it would appear that the facts might be explained by one or more of the following possibilities:

(1) Hydrolysis of more complex, insoluble, organic substances by the action of the caustic alkali used in the extraction.

(2) The presence in the soil of insoluble compounds or adsorption complexes of the non-humic matter with the mineral constituents of the soil, which are split up by alkali or soluble in alkali and decomposed on acidification.

(3) Incorporation of the non-humic matter, or its compounds postulated above, with the humic-clay gel.

That the first possibility cannot account for more than at most a small part of the non-humic matter is shown by the facts that the proportion of non-humic to humic nitrogen is as high in a sodium carbonate extract, as it is in a caustic soda extract, and that in the latter the relative proportion of non-humic matter, so far from rising, shows a tendency to fall as the time of extraction increases. This is well shown by Fig. 1, based on the data recorded in Table I.

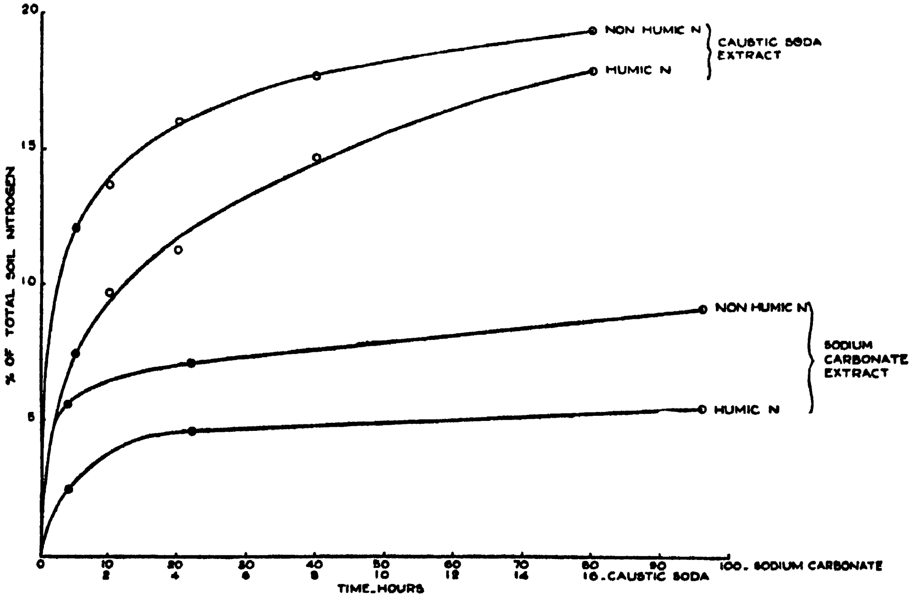

Fig. 1. Extraction of humic and non-humic nitrogen from soil.

It is, however, possible that a part of the non-basic fraction of the non-humic nitrogen may be produced by hydrolysis; for instance, by the splitting off of prosthetic groupings from conjugated proteins. The chemical evidence is not inconsistent with their presence, and they are known to be readily split off by mild alkaline hydrolysis.

Whilst the second possibility alone is an insufficient explanation, it probably plays a definite part in these soils of high clay content. In alkaline solution basic compounds were not held by the soil, but in acid solution they were, although non-basic compounds were not. This suggests that the electro-negative colloidal clay is mainly responsible. About 40 per cent. of the non-humic nitrogen prepared from soil remained unadsorbed in acid solution in contact with fresh or alkali-extracted soil. It is of interest to note that Schmuk (7) found that a podsol yielded a considerable amount of water-soluble nitrogen, which was almost entirely non-basic (not precipitated by phosphotungstic acid).

The third possibility, however, would appear to denote the dominant influence in regard to the state in which the non-humic nitrogen occurs in the soil. Incorporation of the non-humic fraction within the humic-clay gel, whether in the free state or in association with the inorganic colloidal matter, would cause mechanical interference to the extraction. Fig. 1 shows that the non-humic and humic nitrogen came into solution in caustic soda at appreciably the same rate, and that this was still the case when sodium carbonate was used. The non-humic nitrogen came into solution slightly faster at first; the presence of a small amount of non-humic nitrogen coming into solution almost at once would account for this.

The fact that baryta, in which the humic matter is insoluble, nevertheless extracts a part of the non-humic nitrogen shows that diffusion through the humic gel may occur, but that it is not seriously antagonistic to the above view is shown by Table VII. Thus the baryta extract contains mainly the simple more diffusible compounds.

Table VII. *Comparison of extraction of nitrogen by caustic soda and baryta.*

	16 hours soda extract	Equivalent baryta extract
Total non-humic N	21 % T.S.N.	9 % T.S.N.
Precipitated by basic lead acetate	14·5	3·5
N not precipitated by basic lead acetate and not ammonia	3·9	3·5
Ammonia N	2·6	2·0

It is generally supposed that humic matter and colloidal clay form a coating of humic-clay gel around the larger particles. The non-humic matter may be considered to be more or less uniformly incorporated in this coating, partly uncombined and partly associated with the inorganic colloid in the form of iron and aluminium compounds and loose complexes with silica gradually merging into adsorption complexes with clay colloids. Only the simple readily diffusible substances in the interior of the coating, and that part of the more complex non-humic substances which is in the surface layer, could be extracted by solvents in which the humic matter is insoluble. The rest of the non-humic matter would go into solution only in solvents which dissolved the humic matter, and only in proportion to the amount so dissolved. This view, although not readily susceptible to direct proof, is in accordance with the observed facts and offers a satisfactory picture of the state in which the non-humic matter occurs in the soil.

The higher relative proportion of non-humic nitrogen extracted from soils of low organic nitrogen content (Table II) is explained if the clay colloid plays an important part in the retention of the non-humic nitrogen, since all these soils are of high clay content and thus the amount of total colloid varies among the different soils much less than does the amount of colloidal humic matter therein. Even a relatively small proportion of humic matter could bind together a larger amount of colloidal clay in such a way as to protect from attack non-humic substances associated therewith, until the complex was broken up by solution of the humic matter in alkali.

SUMMARY.

1. From an examination of the compounds remaining in solution when humic matter is removed from alkaline extracts of Rothamsted soils by acidification, it is concluded that 30–40 per cent. of the non-humic nitrogen is present in the form of peptides (proteoses, peptones and polypeptides), with 5 per cent. of free amino nitrogen and 12 per cent. of ammonia. The peptides are largely colloidal, and precipitated by basic lead acetate and by phosphotungstic acid. The remaining 40–50 per cent. of the non-humic nitrogen is mainly precipitated by basic lead acetate and is mainly non-basic.

2. The non-humic nitrogen compounds are thought to be incorporated in the humic-clay gel so that for the most part they go into solution only in solvents which dissolve the humic matter, and only in proportion to the amount so dissolved.

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CLI. THE DETERMINATION OF CELLULOSE IN SOIL[1].

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Introductory.

During the course of an investigation into the decomposition of green manures in soil it was necessary to determine the amount of cellulose that underwent changes at the different stages of fermentation. Charpentier [1920] has shown that when cellulose, in the form of filter-paper, is mixed with soil it can be recovered quantitatively by extraction with Schweitzer's reagent, an observation later confirmed by Barthel and Bengtsson [1924]. This method, however, cannot be applied directly to soils mixed with plant materials, as the plant tissues contain, amongst other constituents, hemicelluloses and lignin which interfere with the extraction of cellulose with Schweitzer's reagent. The hemicelluloses are to a certain extent soluble in Schweitzer's reagent and are precipitated with alcohol. Lignin on the other hand acts as a barrier to the complete dissolution of cellulose in Schweitzer's reagent.

Waksman and Tenney [1927] suggested treating the material with a 5 % solution of NaOH for 30 minutes at 15 lbs. pressure in order to remove the hemicelluloses and lignin before extracting the cellulose with Schweitzer's reagent. Solutions of sodium hydroxide of strength greater than 1–2 % are known to attack cellulose, so that any treatment in which the plant material is exposed to the action of alkali of more than 1–2 % strength at high temperatures is bound to give a low yield of cellulose. Bengtsson [1924] proposed treating the soil mixed with plant materials with a solution of sodium bisulphite in hydrochloric acid at a temperature of 100° for 72 hours and then extracting cellulose with Schweitzer's reagent. Apart from the fact that the sulphite process of separating cellulose gives lower yields than the Cross and Bevan method, the period of digestion required for the preliminary treatment of the material makes the process too long.

With a view to the elimination of these disadvantages an attempt was made to find a suitable method for the estimation of cellulose in plant materials mixed with soil.

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[1] This paper is an abridged form of part of a thesis approved for the degree of Ph.D. in the University of London

EXPERIMENTAL.

Some preliminary experiments were carried out on a sample of oat straw whose cellulose content was known. It contained 39·3 % cellulose by Jenkins's method [1930] which gives as good results as the Cross and Bevan method. In all subsequent experiments this figure was taken as the standard with which the results of the various methods tried were compared.

An attempt was first made to see if Schweitzer's reagent could extract all the cellulose from the straw after the hemicelluloses were removed as suggested by Waksman [1927]. One g. portions of straw were treated with 100 cc. of 1 % NaOH and 1 % H_2SO_4 at boiling temperature for half an hour with each reagent. The residue, after thorough washing and drying, was shaken with 100 cc. of Schweitzer's reagent for one hour[1] and the cellulose estimated according to Charpentier's process. The residue was re-extracted twice with Schweitzer's reagent and cellulose precipitated each time as before. The results (Table I) show that even by extracting the material three times, a part of the cellulose only could be recovered. There is a progressive dissolution of cellulose, its complete dissolution being hindered by lignin with which it is supposed to be encrusted.

Table I.

	1st Extraction	2nd Extraction	3rd Extraction	Total
Cellulose on dry matter, %	29·5 27·6	2·94 3·05	2·29 1·66	34·37 32·31

The use of chlorine gas for removing lignin, apart from its other drawbacks, is ruled out where the material under treatment is mixed with soil. The sulphite process of Bengtsson, already referred to, was tried on one g. portions of the oat straw (Table II).

Table II.

	1st Extraction	2nd Extraction	Total
Cellulose % on dry matter	36·7 37·5	0·86 0·50	37·56 38·00

Though the removal of lignin has increased the amount of cellulose that could be extracted with Schweitzer's reagent, yet the yield is not up to the standard. This suggests that a part of the cellulose has been attacked during the preliminary digestion with the bisulphite. It is clear that it is necessary to remove lignin from the plant tissues before the cellulose can be completely dissolved by the Schweitzer's reagent, and yet the reagent employed must be such as to have a minimum effect on the cellulose. Jenkins's method of chlorinating lignin by means of sodium hypochlorite in an alkaline solution suggested itself and was next tried. One g. portions of the straw were subjected to the dilute alkali and acid treatments as before and the residues therefrom were treated twice with sodium hypochlorite as described by Jenkins. The excess of hypochlorite was removed and the residues, after drying, were extracted with 100 cc. of Schweitzer's reagent and cellulose was estimated as before (Table III).

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[1] In some preliminary experiments it was found that shaking for one hour extracted as much cellulose as shaking for 6 or 24 hours.

Table III.

No. of determination	Cellulose % on dry matter
1	40·15
2	39·96
3	40·05
4	39·07
5	40·82
Mean value	40·01 ±0·28

The Schweitzer's reagent is now able to extract the whole of the cellulose at one extraction and the results agree well with the standard.

The straw was now mixed with a light sandy soil, and the mixture subjected to the same treatments as with straw alone. 10 g. of the soil mixed with 1 g. of the straw were used for analysis (Table IV).

Table IV.

No. of determination	Cellulose in soil %	Cellulose in mixture of soil + straw	
		Amount obtained	Amount calculated
1	0·0627	0·3955	
2	0·0546	0·3987	
3	0·0627	0·3915	
4	—	0·3858	
5	—	0·3851	
Mean value	0·0600 ±0·0027	0·3913 ±0·0027	0·4060

The results show that almost the whole of the cellulose added to the soil in the form of straw can be recovered by the method of analysis employed. A part of the cellulose, 0·0147 g. could not be recovered from the mixture. This loss of cellulose which was also observed by Charpentier and others is attributed by them to adsorption by the soil. Whatever the explanation be, it seems certain that a part of the added cellulose, in whatever form it is added, is always retained by the soil and is not recovered by the method of analysis used.

The method was then applied to different plant materials and to mixtures of these plant materials with soil. In all cases 10 g. of the soil together with 1 g. of the plant material were taken for analysis. Table V shows the results of analysis of plant materials alone. In Table VI the figures are given for the same plant materials mixed with the soil.

The results show that the cellulose added to the soil in the form of plant materials of widely different nature and age can be recovered almost quantitatively.

Table V.

	Cellulose on 100 g. dry matter					
	Young tares		Young mustard	Sugar beet tops		Mature mustard
No. of determinations		by Jenkins's method			by Jenkins's method	
1	9·83	9·62	9·42	7·18	7·87	22·90
2	10·48	10·11	8·60	8·12	7·79	23·75
3	9·21	—	—	8·09	—	—
4	9·52	—	—	7·61	—	—
5	9·83	—	—	6·53	—	—
6	9·28	—	—	6·79	—	—
Mean value	9·69	9·87	9·01	7·39	7·83	23·32
Standard error	0·18	—	0·51	0·26	—	0·42

Table VI.

	Cellulose in 10 g. soil + 1 g. plant material, expressed on dry matter			
	Cellulose g.	Average value	Amount calculated	Difference
Young tares + soil	0·0926 0·1015	0·0970	0·1028	0·0058
Young mustard + soil	0·0968 0·0833	0·0901	0·0960	0·0059
Sugar beet tops + soil	0·0731 0·0731 0·0745 0·0731	0·0735	0·0798	0·0063
Mature mustard + soil	0·2375 0·2350	0·2362	0·2391	0·0029

Description of method.

Ten g. of soil are heated in a beaker with 100 cc. of 1 % NaOH and allowed to boil for 20–30 minutes, maintaining a constant volume during heating. The soil is allowed to settle; this may be hastened by making the suspension slightly acid. The upper liquid is filtered off and the residue washed twice with hot water. The material on the filter is transferred to the beaker and the soil heated with 100 cc. of 1 % hydrochloric acid for about 20 minutes, maintaining a constant volume of liquid. After settling, the upper liquid is filtered off and the residue washed free of acid with hot water. The material on the filter-paper is returned to the beaker, 5 cc. of sodium hypochlorite, having 15 % available chlorine, are added and the volume is made up to 100 cc. The liquid must remain alkaline. It is allowed to stand for half an hour in the cold with three or four shakings during the interval. The upper liquid is filtered and the material on the filter returned to the beaker, another 5 cc. of the sodium hypochlorite solution added and the solution made up to 100 cc. and allowed to stand for half an hour with shakings as before. As much as possible of the upper liquid is filtered and to the residue in the beaker a dilute solution of H_2O_2 (10 cc. of 20 vol. H_2O_2 made up to 100 cc.) is gently added till effervescence ceases, excess being avoided. The suspension is allowed to settle, being hastened with HCl if necessary, and the clear liquid is filtered through the same filter-paper. The residue in the beaker is transferred to an evaporating dish and washed thoroughly free of acid with hot water by decantation. The material on the filter-paper is transferred to the dish and the residue evaporated to dryness on a water-bath.

When the residue is completely dry, it is transferred to a suitable bottle for extraction with Schweitzer's reagent. 100 cc. of Schweitzer's reagent are added and the material shaken for one hour. It is then allowed to settle, preferably overnight. More than 50 cc. are filtered, a suction pump being used if necessary. To 50 cc. of the filtrate in a 400 cc. beaker 200 cc. of 80 % alcohol are added, and the precipitate is allowed to settle overnight. It is then filtered by decantation through a suitable filter aided by suction, Jena glass filter-crucible No. 1G4 being very convenient. After the upper liquid has all been filtered, 50 cc. of a mixture of alcohol and HCl (40 cc. of 80 % alcohol *plus* 10 cc. HCl) are added to the precipitate; the mixture is shaken till all the copper hydroxide is dissolved and is then allowed to stand for about an hour. The upper liquid is filtered through the same filter, the precipitate washed free of copper with hot water, and transferred to the crucible, washed further with hot water, then with alcohol and ether in turn as directed by Charpentier. The crucible is dried in an oven for about one hour; the cellulose is transferred to a weighed crucible, weighed, ignited and again weighed. The loss of weight on ignition multiplied by 2 represents the amount of cellulose in 10 g. of the soil.

SUMMARY.

1. A method is described for the determination of cellulose in soil mixed with plant materials. It is essentially a combination of the Jenkins and Charpentier methods. The soil is treated with hot dilute alkali and acid and then with a solution of sodium hypochlorite in the cold. Cellulose is then extracted from the residue with Schweitzer's reagent, precipitated with alcohol and determined by loss of weight on ignition.

2. The results obtained by this method on plant materials alone agree very closely with those given by the hypochlorite method of Jenkins.

3. It is shown that it is necessary to remove the lignin from the plant tissues, otherwise it is not possible to obtain a quantitative yield of cellulose by extraction with Schweitzer's reagent.

4. The method has proved its value in recovering almost the whole of the cellulose added, when different plant materials are mixed with soil. A part of the cellulose is, however, retained by the soil and is not extracted by the Schweitzer's reagent.

5. No attempt has been made to apply the method to different types of soil.

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Nitrite Formation by Soil Bacteria, other than Nitrosomonas.

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Introduction.

The biological nature of nitrification in soil was first established by Schlösing and Müntz (1877). Warington (1878, etc.) at Rothamsted and numerous other investigators elsewhere advanced considerably the knowledge of this process. Winogradsky (1890) first succeeded in isolating two species of bacteria, one capable of oxidising ammonia into nitrite and the other nitrite into nitrate; he further showed that nitrification in soil can take place only through the activity of a very limited group of autotrophic bacteria possessing the peculiar property of growing exclusively in an inorganic medium having a very pronounced alkaline reaction.

In 'Nature' Cutler (1930) reported that several different bacterial strains capable of oxidising various ammonium salts to nitrite had been isolated from different sources. A description of some of these bacteria occurring in soil, together with their physiological reactions, is given in this paper.

As a result of investigations on the growth of bacteria on silica gel plates, some species were found which were capable of oxidising ammonium salts to nitrite; but which did not resemble the nitrosomonas group of organisms in that they grew freely on nutrient agar medium containing lemco and peptone. This was interesting, especially in view of the observation made by Winogradsky (1890, 1891) that the most striking characteristic of his nitrite producers, *Nitrosomonas* or *Nitrosococcus*, was their marked repugnance to organic substances.

There are, however, scattered references in the literature to organisms capable of oxidising ammonia to nitrite and nitrate, and of growing in media containing organic as well as inorganic nutrient substances.

Stützer and Hartleb in 1897 isolated *Nitrosomonas* by using magnesium-ammonium-phosphate agar plates. Silicic acid gel was also used by Stützer (1901), but did not give satisfactory results.

Beddies in 1899 reported the isolation of nitrifying organisms; these were not very sensitive to high concentrations of organic matter and the presence of humus in the medium aided the growth.

Fremlin in 1903 isolated a nitrite-forming organism, and showed that this bacterium would grow on bouillon. In 1914, using agar, he found that the presence of broth or urine increased the activity of this micro-organism ; and recently (1930) he has again shown that it is capable of growing in the presence of organic substances.

Makrinoff (1909) isolated a nitrite-forming organism by employing gypsum plates and found that the presence of soil did not prove toxic ; a result also obtained by Gibbs (1909) when cultivating *Nitrosomonas*.

Mishustin (1926) found two spore-forming bacteria in soil which produced nitrites in media containing organic nitrogenous compounds, but not in inorganic media containing ammonium salts.

Runov (1926) obtained two species from enrichment cultures, one of these produced nitrites from organic nitrogen compounds and from ammonia in the presence of organic substances. Neither organism grew on Winogradsky's medium and the author concluded that there were many bacteria in nature capable of forming nitrites in media containing various organic substances.

The diagonistic characters given in these cases were, however, insufficient for identification purposes.

Experimental.

In the course of work on the isolation of *Nitrosomonas*, numerous bacterial colonies were picked from silica gel plates for further investigation. These were inoculated into Winogradsky's liquid medium containing excess of ammonium sulphate but no magnesium carbonate.* Periodic nitrite determination by the Griess-Ilosva method showed that these organisms were capable of oxidising ammonium sulphate into nitrite, and the quantities of nitrite formed compared very favourably with those found in a similar culture inoculated with fresh soil.

Portions of these enrichment cultures were plated on Thornton's agar and white colonies appeared after 4 days. A number of these colonies were picked off and inoculated on to Thornton's agar slopes, where a copious growth soon took place. Six strains, A, B, C, D, E and F were selected for further work.

In order to test whether the presence of sugar exerted any effect on the production of nitrite by these organisms the following mineral salt medium was made :—

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* It was found from preliminary experiments that magnesium carbonate was not essential to the growth or activity of these organisms.

	Per cent.
$(NH_4)_2SO_4$	0·1
NaCl	0·06
$CaCl_2$	0·002
$MgSO_4 7H_2O$	0·0005
KH_2PO_4	0·03

The p_H value after autoclaving was brought to 7·3 by the addition of NaOH and 0·1 per cent. sucrose was added to some of the medium.

50 c.c. portions of these two media were distributed into an equal number of 250 c.c. Erlenmeyer flasks which were inoculated with the bacteria.

The amounts of nitrite produced in each of the cultures are shown in Table I.

Table I.—Nitrite Nitrogen in milligrammes per litre produced by the different species in mineral salts medium—both in the presence and absence of sugar.

Days.	Sugar.	A.	B.	C.	D.	E.	F.	Control.
2	+	0·13	Trace	Nil	Nil	Trace	0·13	Nil
	—	0·13	,,	,,	Trace	,,	Nil	,,
5	+	0·25	0·15	0·15	Nil	0·13	Trace	,,
	—	0·13	Trace	Nil	,,	Nil	Nil	,,
8	+	0·5	0·25	,,	0·13	0·25	0·25	,,
	—	0·13	0·13	Trace	Nil	0·13	Trace	,,
12	+	0·75	0·75	0·25	0·25	0·25	0·5	,,
	—	0·25	0·5	Trace	Nil	Nil	Nil	,,
17	+	1·25	1·0	0·50	0·25	0·5	0·5	,,
	—	0·25	0·5	0·25	0·25	Nil	Trace	,,
23	+	1·0	1·0	0·75	0·50	1·0	1·0	,,
	—	0·50	0·75	0·25	0·25	0·25	Nil	—
28	+	0·5	0·5	0·25	0·75	0·75	1·0	—
	—	0·5	0·5	Nil	0·25	0·50	0·5	—

It is evident that in the presence of sugar these organisms produced more nitrite than in its absence, which is of interest considering that no growth occurs in sugar solutions (see p. below). In all the following investigations, therefore, the mineral salt medium containing 0·1 per cent. sucrose was used unless otherwise stated.

To ensure that only pure cultures were being investigated repeated platings and pickings off of single colonies were made; and the pure strains finally obtained were known as A_1, A_2, B_2, C_{21}, C_{22}, D_{33}, E and F_{11}.

The nitrite produced by each of these strains from ammonium phosphate, chloride, lactate, acetate and sulphate is shown in Table II. The nitrogen equivalent of each of the added salts amounted to 0·02 gm. per 100 c.c. medium.

Table II.—Nitrite formed by species A_1. A_2, B_2, C_{21}, C_{22}, D_{33}, E and F_{11} from various ammonium salts. (Nitrite-nitrogen in milligrammes per litre.)

Ammonium salts.	Bacteria.	Days.						
		2.	4.	6.	9.	11.	13.	16.
Phosphate	A_1	0·125	Nil	Nil	Trace	Trace	Trace	Trace
	A_2	Nil	,,	Trace	0·125	0·25	0·25	0·125
	B_2	,,	Trace	,,	Nil	Nil	Nil	Nil
	C_{21}	,,	Nil	0·25	0·5	0·25	Trace	Trace
	C_{22}	,,	,,	Nil	Nil	Nil	,,	Nil
	D_{33}	,,	,,	Trace	0·5	0·5	0·5	0·125
	E	,,	,,	0·125	0·25	0·25	Nil	Nil
	F_{11}	,,	0·125	0·25	0·5	0·5	,,	0·125
Chloride	A_1	,,	Nil	Nil	0·25	0·5	Trace	Trace
	A_2	,,	0·125	0·25	0·5	0·25	0·75	0·5
	B_2	,,	Trace	Nil	Nil	Nil	Trace	0·125
	C_{21}	,,	,,	,,	,,	,,	,,	Nil
	C_{22}	Trace	Nil	,,	,,	Trace	0·25	0·25
	D_{33}	Nil	0·125	0·125	0·25	0·25	0·25	0·25
	E	,,	Nil	Nil	Nil	Nil	Nil	Trace
	F_{11}	,,	,,	,,	Trace	,,	,,	Nil
Lactate	A_1	,,	Trace	0·125	0·75	0·75	0·75	0·5
	A_2	,,	0·125	0·5	0·5	0·5	0·75	0·5
	B_2	,,	Trace	0·25	0·5	0·25	Nil	Nil
	C_{21}	,,	,,	Trace	Nil	Nil	,,	,,
	C_{22}	,,	Nil	0·125	Trace	Trace	Trace	Trace
	D_{33}	Trace	0·125	0·5	0·5	0·25	0·25	0·25
	E	Nil	Nil	Nil	Nil	Nil	Nil	Nil
	F_{11}	,,	,,	,,	,,	,,	,,	Trace
Acetate	A_1	,,	,,	Trace	,,	,,	,,	Nil
	A_2	Trace	,,	0·125	0·25	0·25	0·25	0·125
	B_2	Nil	,,	Nil	Nil	Nil	Nil	Nil
	C_{21}	,,	,,	,,	Trace	Trace	,,	,,
	C_{22}	,,	,,	0·125	Nil	Nil	,,	,,
	D_{33}	,,	,,	Nil	,,	0·25	0·25	Trace
	E	,,	,,	,,	,,	Trace	Trace	Nil
	F_{11}	,,	0·125	0·125	,,	Nil	Nil	,,
Sulphate	A_1	,,	Trace	Nil	0·25	Trace	,,	,,
	A_2	,,	0·125	0·125	0·25	0·25	,,	,,
	B_2	,,	Nil	0·125	0·25	0·5	0·5	0·25
	C_{21}	Trace	Trace	Nil	0·125	Trace	Trace	Nil
	C_{22}	Nil	Nil	,,	0·125	0·125	Nil	,,
	D_{33}	Trace	,,	,,	0·5	0·5	0·25	Trace
	E	,,	0·125	0·25	0·75	0·5	Nil	,,
	F_{11}	Nil	Trace	0·25	0·5	0·25	,,	,,

It will be seen that ammonium phosphate, chloride, lactate and sulphate can be oxidised into nitrite by these bacteria, but with ammonium acetate the oxidation is not very marked. The yields of nitrite are greatest with lactate and sulphate of ammonia, the former giving somewhat better results than the latter excepting in the cases of E and F_{11}.

It is interesting to note that in the majority of the cultures the amounts of nitrite diminished gradually, and the following experiments were carried out in order to test if the organisms were capable of utilising nitrite.

Sulphate of ammonia was replaced by sodium nitrite in the mineral salt medium containing 0·1 per cent. sucrose. The amount of nitrite in the medium corresponded to 1·8 mgm. of nitrogen per litre, and 50 c.c. portions of the medium were distributed into 250 c.c. Erlenmeyer flasks which were subsequently inoculated.

The results of the nitrite estimations are given in Table III.

Table III.—Nitrite Utilisation by A_1, A_2, B_2, C_{21}, C_{22}, D_{33}, E and F_{11}. (NO_2-N) originally present: 1·8 mgm. per litre.)

Days.	NO_2-N in milligrammes per litre.								
	A_1.	A_2.	B_2.	C_{21}.	C_{22}.	D_{33}.	E.	F_{11}.	Control.
0	1·8	1·8	1·8	1·8	1·8	1·8	1·8	1·8	1·8
2	1·8	—	—	1·8	1·8	1·8	1·8	Trace	1·8
3	—	Trace	Trace	—	—	—	0·05	—	1·8
4	0·8	—	—	1·8	0·6	1·8	—	Nil	1·8
6	—	Nil	Nil	—	—	—	Trace	—	1·8

No nitrate was formed in any of the cultures, so it was clear that none of the eight organisms could oxidise nitrite into nitrate.

It will be observed that A_2, B_2, E and F_{11} utilised the whole of the nitrite originally present in the medium. A_1 and C_{22} appeared to assimilate it partially, while C_{21} and D_{33} did not absorb nitrite at all in the time under consideration.

It is well known that nitrate-forming bacteria can function only in a medium containing nitrite, but it is very curious that nitrite-forming organisms should absorb any nitrite that they themselves have produced from ammonium salts. It seems probable therefore that, although essentially nitrite formers, they also can use nitrite for their metabolism. Certain soil Actinomycetes are capable of utilising nitrite when it is present in low concentrations.

With a view to ascertaining the effect of soil on the nitrifying powers of these bacteria, the production of nitrite was studied in sterilised soil treated with sulphate of ammonia and inoculated with each species.

For this purpose air-dried Barnfield soil receiving farmyard manure was employed. The soil was passed through a 2-mm. sieve and sterilised by heating in an air oven for 1 hour at 190° C. 300 gm. portions of this soil were moistened with 60 c.c. of an aqueous solution of 0·1 per cent. ammonium sulphate and were distributed into 2-litre conical flasks previously sterilised. The soil was inoculated with 1 c.c. of bacterial suspension. 20 gms. of soil were periodically withdrawn, shaken up with 100 c.c. of distilled water and the nitrite estimated in the extract by the Griess-Ilosva method.

The nitrite formed in each case is shown in Table IV.

Table IV.—Production of Nitrite from Ammonium Sulphate in Soil by Bacteria A_1, A_2, B_2, C_{21}, C_{22}, D_{33}, E and F_{11}. NO_2-nitrogen in milligrammes per kilo of soil.

Days.	A_1.	A_2.	B_2.	C_{21}.	C_{22}.	D_{33}.	E.	F_{11}.	Control.
2	1·0	Nil	Nil	Nil	Nil	Trace	Trace	Nil	Nil
3	1·7	,,	,,	Trace	Trace	0·6	0·6	Trace	,,
4	—	—	—	0·7	—	0·6	0·6	,,	,,
5	2·0	Trace	Trace	—	0·25	—	—	0·8	,,
6	1·7	,,	,,	1·3	Trace	1·3	1·3	1·7	,,
8	0·9	0·6	Nil	Trace	Nil	1·9	1·7	1·7	,,
9	—	—	—	,,	—	1·9	1·3	—	,,
10	—	—	—	,,	—	Trace	1·3	—	,,
11	0·4	0·5	Nil	0·4	Trace	,,	1·3	1·0	,,

It is seen that A_1, A_2, C_{21}, D_{33}, E and F_{11} are definitely capable of oxidising ammonium sulphate into nitrite in soil. In none of the cultures was nitrate found.

Effect of Aeration.

Aeration has a favourable influence upon most soil organisms, and it may be assumed to be particularly beneficial to oxidising organisms. The influence of aeration on some of the strains isolated was therefore investigated.

A_2 and D_{33} were selected for this purpose, since they showed such marked differences in their assimilation of nitrite. Two flasks each containing 100 c.c. of the mineral salt medium containing 0·1 per cent. ammonium sulphate were inoculated with A_2 and D_{33} respectively. Air from which the carbon dioxide and nitrite had been removed was aspirated through the cultures at the rate of about 6 litres in 24 hours. The vessels were kept immersed in a thermostat maintained at 23° C. and samples of the cultures for nitrite and bacterial determinations were taken by means of sterilised rubber tubing.

The results obtained are given in Table V.

Table V.—Effect of Aeration on the Oxidation of Ammonia to Nitrite by Strains A_2 and D_{33}. (Nitrite-nitrogen is expressed in milligrammes per litre and the bacterial numbers in millions per cubic centimetres.

Days.	A_2.		D_{33}.	
	NO_2-N.	Bacterial numbers.	NO_2-N.	Bacterial numbers.
0	Nil	31·0	Nil	44·0
2	0·62	48·6	0·5	35·7
5	1·0	78·6	1·5	25·7
7	0·5	Innumerable	1·4	26·8
8	0·25	,,	1·25	32·0

Aeration evidently stimulated the production of nitrite especially during the first few days, since the amounts of nitrite given in Table V are greater than those given in Table II. The culture inoculated with A_2 showed a rapid increase in the bacterial numbers, whereas those in D_{33} tended to diminish. In A_2 the nitrite diminished with the age of the culture, but with D_{33} no such diminution was noticed; this was in accordance with the results already obtained (Table III), that while A_2 assimilated nitrite vigorously, D_{33} did not.

Experiments with Mixed Cultures.

Investigations were next carried out with mixed cultures in which the ammonia was produced from asparagin by the ammonifying bacterium "YB" isolated from soil in this laboratory.

The composition of the medium was :—

	Per cent.
K_2HPO_4	0·1
$MgSO_4\,7H_2O$	0·02
$CaCl_2$	0·01
NaCl	0·01
$FeCl_2$	Trace
Asparagin	0·05

The nitrogen equivalent of the asparagin added was 53 mgm. per litre, and the production of ammonia and nitrite was followed. The ammonia was estimated by Nessler's method; and the results are given in Table VI.

Table VI.—Production of Ammonia and Nitrite from Asparagin by Mixed Bacterial Cultures. (Ammonia and nitrite expressed as nitrogen in milligrammes per litre.)

Strains.	3 days.		6 days.	
	Nitrite.	Ammonia.	Nitrite.	Ammonia.
YB + A_2	0·5	40	0·85	50·0
A_2	Trace	30	Nil	37·5
YB + C_{21}	0·4	12·5	0·4	20·0
C_{21}	0·25	Trace	Nil	15·0
YB + D_{33}	0·8	37·5	0·75	37·5
D_{33}	0·4	12·5	0·25	10·0
YB + E	0·4	25	0·4	25·0
E	0·2	12·5	0·25	15·0
YB + F_{11}	0·5	25	0·5	25·0
F_{11}	Trace	Trace	0·13	15·0

These experiments show that A_2, C_{21}, D_{33}, E and F_{11} can all produce ammonia from asparagin though not to the same extent as YB. The nitrite formed from the ammonia that they themselves produce is, however, very low in comparison with the nitrite formed in association with YB. These nitrite organisms when alone can take an active part in breaking up the asparagin; but during the process they possibly expend most of their energy and are able only to take a feeble part in oxidising this ammonia further into nitrite. When, on the other hand, they are associated with YB, the asparagin-ammonia transformation being mainly brought about by the latter bacteria, the nitrifiers are free to oxidise the ammonia produced into nitrite. Again there was no trace of nitrate in any of the cultures.

Description of Strains.

The characterisation of the strains according to the group number as arranged by the Society of American Bacteriologists is given as follows:—

Group number.	Characterisation.	
200	Endospores not produced	All.
10	Aerobic (strict)	All.
1	Gelatine liquefied	A_2, C_{22}, D_{33}, F_{11}.
2	Gelatine not liquefied	A_1, B_2, C_{21}, E.
0·4	No growth with dextrose*	All.
0·04	No growth with lactose	All.
0·004	No growth with saccharose	All.
0·0002	Nitrates reduced without gas	A_1, A_2, B_2, C_{21}, E, F_{11}.
0·0003	Nitrates not reduced	C_{22}, D_{33}.
0·00000	Non-chromogenic	All.
0·000003	Diastatic action on starch absent ..	All.
0·0000004	No growth with glycerine	All.

Total Group Number.

A_1	212·4442034
A_2	211·4442034
C_{21}	212·4442034
C_{22}	211·4443034
D_{33}	211·4443034
E	212·4442034
F_{11}	211·4442034

The characteristics of the organisms not used for finding the group number are as follows :—

Agar Slopes. 3 *days old.*—The growth of all the organisms was abundant; echinulate, raised, smooth, glistening and opaque; viscid and no odour; medium unchanged.

Agar Stabs. 3 *days old.*—All uniform, filiform, medium unchanged.

Gelatine Stabs.—A_1, B_2, C_{21}, E, line of puncture filiform; no liquefaction. A_2, C_{22}, D_{33}, F_{11}, surface growth, liquefaction.

Colonies on Thornton's Agar.—In all cases the growth is rapid, surface smooth, internal structure amorphous. There are minor differences in the character of the edge and the elevation, the edge in A_1, A_2, B_2, C_{22}, D_{33} and E being entire, while that of C_{21} and F_{11} is undulating. The elevation of the colonies of all the strains is convex, except in the case of A_2, C_{22} and E, where it is flat.

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* Besides the sugars mentioned here no growth was obtained with mannitol, maltose laevulose and galactose.

Morphology and Staining.—These characters are given in Table VII.

Table VII.

Strains.	Gram.	Shape.	Dimensions in μ.	Acid fast.	Motility.
A_1	+	Rods	1·90 × 0·85	—	—
A_2	+	,,	1·39 × 0·77	—	—
B_2	+	,,	1·89 × 0·82	—	—
C_{21}	+	,,	1·84 × 0·81	—	—
C_{22}	+	,,	1·43 × 0·71	—	—
D_{33}	+	,,	1·80 × 0·80	—	—
E	+	,,	1·73 × 0·75	—	—
F_{11}	—	Coccus	0·93	—	—

It will be seen that some of these strains have a common group number and similar morphological characteristics, and therefore they may be classified into four groups :—

I.—A_1, B_2, C_{21} and E.
II.—A_2.
III.—C_{22} and D_{33}.
IV.—F_{11}.

Reference to Table II shows that the oxidising power is not similar in what are probably the same strains ; but it must be remembered that in these experiments uniform inoculations were not made.

From the foregoing data it will be seen that in sharp contrast with Winogradsky's nitrite-forming bacteria the organisms described are capable of growing on nutrient agar and in the presence of sugar. They can produce nitrite within a wide range of p_H values varying from 4·8 to 7·3, whereas *Nitrosomonas* or *Nitrosococcus* can function only in a distinctly alkaline medium. Morphologically, too, they are different from *Nitrosomonas*, which first forms a zooglea and then breaks up into swarming, ellipsoidal, motile bacteria.

Summary.

Four species of non-spore-forming bacteria capable of oxidising ammonia into nitrite have been isolated from Rothamsted soil and all differ widely from *Nitrosomonas* or *Nitrosococcus.*

These organisms are able to carry out this reaction in artificial media as well as in soil, and some are able to assimilate nitrite.

Rapid growth takes place on nutrient agar, and the presence of 0·1 per cent. sucrose stimulates nitrite production.

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A COMPARISON OF TWO AGAR MEDIA FOR COUNTING SOIL MICRO-ORGANISMS.

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IT was found in the study carried out by Fisher, Thornton and Mackenzie (2) on the accuracy of the plate method for estimating bacterial numbers, that at least one medium, viz. the mannite-asparagine agar devised by Thornton (5), will, even with the mixed population of the soil, give results which are in fair agreement with theoretical requirements, namely that the numbers of bacterial colonies on the plates shall depend solely upon the numbers of bacterial cells in the inoculum which are able to develop on the said medium. When this is the case, values of the index of dispersion χ^2, calculated by the formula

$$\chi^2 = \frac{S(x - \bar{x})^2}{\bar{x}}$$

(where x is the number of colonies on each plate, $\bar{x}$ the mean), are distributed according to a definite law (Fisher (1)), and the average of these values is equal to the number of degrees of freedom, *i.e.* one less than the number of parallel plates.

In their comments upon a paper by Smith and Worden (4), in which the numbers of bacteria in a soil were determined on three agar media, Thornton and Fisher (6) point out that only one of these media—the mannite-asparagine agar—has been tested for its χ^2 distribution. It would therefore be desirable to extend this test to other agar media. The author found an opportunity of doing this when carrying out a number of bacterial counts in soils during some experiments on the decomposition of farmyard manure and dead microbial protoplasm. The material also provided an opportunity of testing the χ^2 distribution for counts of actinomycetes colonies. The soils used were:

1. Heavy clay soil, rich in organic matter and of neutral reaction, from Rothamsted grass plots; with and without addition of farmyard manure and straw.
2. Light sand soil, poor in organic matter and of faintly acid reaction,

from Woburn Experimental Farm; with and without addition of lime, farmyard manure, and straw.

3. Heavy clay soil, poor in organic matter and of faintly acid reaction, from unfertilised plot on Hoos Field, Rothamsted.

4. Another sample from the same field.

5. Heavy loam, rich in organic matter, from a plot of garden soil outside the laboratories of Rothamsted Experimental Station.

The soils were kept in a moist condition in the laboratory for periods up to 500 days, some at room temperature and some at 25° C. The two agar media, on which counts were carried out, were:

1. Mannite-asparagine agar (Thornton (5)).

2. A modification of the dextrose-casein agar mentioned by Waksman (7): dextrose, 2·0 gm.; casein dissolved in 0·1 N NaOH, 0·2 gm.; K_2HPO_4, 0·5 gm.; $MgSO_4$, 0·2 gm.; $FeCl_3$, trace; agar, 15·0 gm.; distilled water, 1000 c.c. of pH 6·4–6·6.

Counts on these two media were made in eighty instances, mostly from soils 1 and 5. Plates were poured on both media from the same soil suspension, the concentration of which ranged from 1:100,000 to 1:2,000,000 according to the numbers of micro-organisms in the soil. The plates were incubated for the same period (10 days) in the same incubator (20° C.). The number of parallel plates ranged from 4 to 10, and was in most cases 5 or 6. Plates obviously spoiled by fungi or fluorescent bacteria were discarded. The average numbers of bacterial colonies per plate ranged from 44 to 460 on mannite agar, and from 54 to 796 on dextrose agar. The corresponding limits for the numbers of actinomycetes colonies were 2 and 110 on mannite agar, 6 and 97 on dextrose agar. The number of bacterial colonies in 74 cases out of 80 was higher on dextrose than on mannite agar, in several cases 2 to 4 times as high. The significance of these differences was tested by calculating the following statistics (Fisher (1)):

$$\text{Standard deviation} \quad s = \sqrt{\frac{S(x_1 - \bar{x}_1)^2 + S(x_2 - \bar{x}_2)^2}{n_1 + n_2}},$$

and

$$t = \frac{\bar{x}_1 - \bar{x}_2}{s} \sqrt{\frac{(n_1 + 1)(n_2 + 1)}{n_1 + n_2 + 2}},$$

where x_1 and x_2 = numbers of colonies on individual parallel plates of the two media, $\bar{x}_1$ and $\bar{x}_2$ = means of numbers of colonies, n_1 and n_2 = degrees of freedom (number of parallel plates − 1).

From Fisher's table of t the probability, P, of the difference being accidental was found. If P was less than 0·05 the difference was considered significant.

Table I. *Comparison between numbers of colonies of bacteria and actinomycetes on mannite-asparagine agar* (MA) *and dextrose-casein agar* (CA).

	Bacteria		Actinomycetes	
Ratio $\frac{\text{Colonies } CA}{\text{Colonies } MA}$	Occurrences	Cases of significant difference	Occurrences	Cases of significant difference
0·60–0·79	0	0	3	1
0·80–0·99	6	1	6	0
1·00–1·19	20	8	21	3
1·20–1·39	20	19	18	13
1·40–1·59	13	13	11	11
1·60–1·79	8	8	6	6
1·80–1·99	4	4	6	6
2·00–2·19	3	3	2	2
2·20–2·39	3	3	1	1
2·40–2·59	1	1	0	0
2·60–2·79	1	1	2	2
2·80–2·99	0	0	0	0
3·00–3·19	0	0	2	2
3·20–3·59	0	0	0	0
3·60–3·79	0	0	1	1
3·80–4·00	1	1	0	0
Above 4·00	0	0	1	1
Total	80	62	80	49
Lowest ratio	0·85		0·74	
Highest ratio	3·94		4·50	

Table I shows that only in one case was the number of bacteria significantly higher on mannite agar than on dextrose agar, whereas the reverse occurred in 61 cases. The number of actinomycetes, too, was significantly higher on mannite agar in only one case, but significantly higher on dextrose agar in 48 cases.

The values of χ^2 for bacteria and actinomycetes obtained with mannite-asparagine agar are shown in Table II. Since we were dealing with several sets with varying numbers of parallel plates, use was made of the method shown by Fisher (1) for testing the reliability of the counts under those conditions. The totals of observed χ^2 and degrees of freedom (n) are summed up; if the difference $\sqrt{2\chi^2} - \sqrt{2n-1}$ exceeds $+2$ or is less than -2, the value of χ^2 is not in agreement with expectation, *i.e.* the variability is either excessively high or abnormally low.

The χ^2 for bacteria was not as a whole outside the range of expectation (as also found by Fisher, Thornton and Mackenzie (2)), although there seems to have been a tendency to excess in the few 4-plate sets.

The χ^2 for the actinomycetes, on the other hand, showed a definitely subnormal variation, which, according to Fisher, Thornton and Mackenzie (2), is usually due to some defect in the medium.

Table II. *Values of χ^2 in counts of bacteria and actinomycetes on mannite-asparagine agar.*

Plates in set ($n+1$)	Numbers of sets (S)	$S(n)$	Totals of χ^2 Bacteria	Totals of χ^2 Actinomycetes
4	3	9	24·33	7·71
5	20	80	79·67	66·76
6	43	215	237·55	167·43
7	8	48	60·51	26·88
8	4	28	33·74	26·27
9	2	16	8·06	5·04
Total	80	396	443·86	300·09
Difference $\sqrt{2\chi^2} - \sqrt{2n-1}$			+1·67	−3·62

A much larger number of counts was carried out on the dextrose-casein agar in order to get sufficient data for a study of the distribution of χ^2 on this medium, which has not previously been tested in this respect. The counts included in all:

2 sets with 3 parallel plates.
33 „ 4 „
270 „ 5 „
38 „ 6 „
11 „ 7 „
2 „ 8 „
1 „ 9 „

The distribution of χ^2 in the 270 5-plate sets is shown in Table III. (Values of χ^2 and expected frequencies taken from Fisher (1), *Table III.*)

The χ^2 for the bacterial counts showed upon the whole a good agreement with expectation, with some deficiency in the lower and some excess in the higher classes of variation. This medium thus seemed to be much like the mannite-asparagine agar in this respect, since it had only a slight tendency to give excessive variation; this is also the case with mannite-asparagine agar, as shown by Fisher, Thornton and Mackenzie, and as seen from Table VI. The χ^2 from counts of actinomycetes agreed almost perfectly with expectation in the lower and the very high classes. In the classes of χ^2 from 4·9 to 9·5 there was a deficiency, and in the classes near the mean ($\chi^2 = 4$, equal to the number of degrees of freedom) there was a corresponding excess.

The counts in the rest of the sets gave χ^2 values of a similar character, as shown in Table IV. Here again the observed values of χ^2 for both bacteria and actinomycetes are, as a whole, in good agreement with expectation.

Table III. *Distribution of χ^2 in counts of bacteria and actinomycetes on dextrose-casein agar (5-plate set).*

χ^2	Expected	Observed	
		Bacteria	Actinomycetes
0	3	0	1
0·297	3	2	3
0·429	7	6	11
0·711	14	8	14
1·064	27	19	28
1·649	27	17	24
2·195	54	57	62
3·357	54	64	69
4·878	27	21	20
5·989	27	29	16
7·779	14	20	9
9·488	7	16	7
11·668	3	8	3
13·279	3	3	2
Total	270	270	269

Table IV. *Values of χ^2 in counts of bacteria and actinomycetes on dextrose-casein agar.*

Plates in set $(n+1)$	Number of sets (S)	$S(n)$	Totals of χ^2	
			Bacteria	Actinomycetes
3	2	4	4·91	2·37
4	33	99	148·90	137·15
6	38	190	192·39	142·76
7	11	66	73·45	50·37
8	2	14	7·19	13·48
9	1	8	3·12	4·05
Total	87	381	429·96	350·18
Difference $\sqrt{2\chi^2} - \sqrt{2n-1}$			+1·73	−1·12

The data treated here have been obtained from several soils of different character, and moreover the additions of various organic materials to the soils have given rise to development of widely different microfloras in the different soils. In Tables III and IV, as well as in Table II, all the data have been combined, with the possibility that some of the series may have shown excessive, others subnormal variation, and that these abnormalities may have compensated each other in the final result. To check this, the figures in Table V have been calculated. For 33 different series of counts on casein agar (6 without and 27 with addition of organic material) the values of χ^2 and n have been summed up; when $S(n)$ exceeded 30, the difference $\sqrt{2\chi^2} - \sqrt{2n-1}$

has been calculated, and when $S(n)$ was less than 30, the value of P, taken from Fisher's Table III, has been recorded. When this value exceeded 0·95 or was less than 0·05, the variation was considered abnormal.

Table V. *Values of χ^2 in different soils. Counts on dextrose-casein agar.*

Soil no.	Addition		Number of sets (S)	$S(n)$	Total χ^2	Difference $\sqrt{2\chi^2}-\sqrt{2n-1}$
		Soils without organic matter.				
2	Nothing	Bact.	13	51	55·00	0·44
		Act.	13	51	51·86	0·13
2	CaCO$_3$	Bact.	14	52	51·65	−0·01
		Act.	14	52	66·55	1·39
3	Nothing	Bact.	11	43	40·31	−0·24
		Act.	11	43	31·80	−1·24
4	Nothing	Bact.	8	32	39·19	0·92
		Act.	8	32	27·92	−0·46
1	Nothing	Bact.	17	89	102·20	1·00
		Act.	17	89	79·93	−0·65
5	Nothing	Bact.	16	70	92·58	1·78
		Act.	16	70	56·82	−1·13
	Total	Bact.	—	337	381·13	1·77
		Act.	—	337	314·19	−0·84
		Soils with organic matter.				
2	Farmyard manure	Bact.	13	51	55·02	0·44
		Act.	13	51	63·64	1·22
2	Do. + (NH$_4$)$_2$SO$_4$	Bact.	13	50	47·73	−0·18
		Act.	13	50	59·54	−0·95
2	Do. + Do. + oat straw	Bact.	12	46	45·79	−0·03
		Act.	12	46	43·18	−0·26
2	CaCO$_3$ + manure	Bact.	12	48	*84·52*	*3·25*
		Act.	12	48	38·76	−0·95
2	Do. + Do. + (NH$_4$)$_2$SO$_4$	Bact.	13	52	47·83	−0·37
		Act.	13	52	43·80	−0·79
2	Do. + Do. + Do. + oat straw	Bact.	14	54	64·86	1·05
		Act.	14	54	53·51	−0·02
1	Farmyard manure	Bact.	17	78	86·64	0·71
		Act.	17	78	*51·86*	*−2·27*
1	Do. + oat straw	Bact.	17	77	99·74	1·75
		Act.	17	77	*51·11*	*−2·26*
3	Farmyard manure	Bact.	11	47	38·08	−0·91
		Act.	11	47	38·95	−0·81
3	"Edelmist"	Bact.	11	44	48·39	0·51
		Act.	11	44	33·18	−1·18
4	Farmyard manure	Bact.	8	32	33·83	0·30
		Act.	8	32	27·37	−1·53
4	Do. dried	Bact.	8	32	*63·10*	*3·29*
		Act.	8	32	32·88	0·17
4	Synthetic farmyard manure	Bact.	8	32	49·04	1·97
		Act.	8	32	31·19	−0·03

Table V (*cont.*).

Soil no.	Addition		Number of sets (S)	S (n)	Total χ^2	Difference $\sqrt{2\chi^2}-\sqrt{2n-1}$
5	Mycelium of *Trichoderma*	Bact.	8	33	39·17	0·81
		Act.	8	33	*63·76*	*3·23*
5	Mycelium of *Zygorhynchus*	Bact.	9	38	48·38	1·18
		Act.	9	38	36·34	−0·13
5	Mycelium of *Act. griseus*	Bact.	9	39	52·36	1·45
		Act.	9	39	39·47	0·11
5	Mycelium of *Asp. fumigatus*	Bact.	9	38	28·17	−1·16
		Act.	9	38	51·68	1·51
5	Mycelium of *Polyporus* sp.	Bact.	10	47	52·70	0·63
		Act.	10	47	*25·65*	*−2·48*
5	Protoplasm of *Bac. megatherium*	Bact.	7	31	31·44	0·12
		Act.	7	31	19·32	−1·60
Sets with S (n) less than 30:						P
5	Protoplasm of *Bacterium* sp.	Bact.	7	25	27·22	0·50–0·30
		Act.	7	25	20·01	0·80–0·70
5	Mycelium of *Stachybotrys*	Bact.	5	17	12·91	0·80–0·70
		Act.	5	17	12·87	0·80–0·70
5	Mycelium of *Mycogone nigra*	Bact.	6	24	32·90	0·20–0·10
		Act.	6	24	18·61	0·80–0·70
5	Mycelium of *Coprinus* sp.	Bact.	7	26	23·32	0·70–0·50
		Act.	7	26	20·81	0·80–0·70
5	Chitin	Bact.	5	23	20·12	0·70–0·50
		Act.	5	23	13·55	0·95–0·90
5	Keratin	Bact.	4	16	19·38	0·20–0·10
		Act.	4	16	*6·33*	*0·99–0·98*
4	Chitin	Bact.	4	15	*33·64*	Below *0·01*
		Act.	4	15	8·10	0·95–0·90
4	Keratin	Bact.	3	12	5·69	0·95–0·90
		Act.	3	12	10·93	0·70–0·50
	Total	Bact.	—	1037	1181·97	3·09
		Act.	—	1037	916·43	−2·72
Omitting the series with abnormal variation:						
	Total	Bact.	—	942	1000·71	1·35
	,	Act.	—	786	717·72	−1·75

The table shows that in the soils without addition of organic matter the values of χ^2 for both bacteria and actinomycetes were within the range of expectation in the individual series as well as in the total. In the series with organic matter the χ^2 for the bacteria were within the range of expectation in 23 cases out of 26; in the remaining 3, soil No. 2 with lime and farmyard manure, and soil No. 4 with chitin and with dried farmyard manure, there was a distinctly excessive variation. This appears very clearly in Table V, where all the values of χ^2 and n from series with organic matter are summed up and the difference calculated. If the three excessive series are omitted, the difference is reduced sufficiently to come within the range of expectation. The figures for actinomycetes showed only 1 case of excessive variation (soil 5 with addition of *Trichoderma* mycelium), but 4 cases of subnormal variation (soil 1 with manure and with manure + straw, soil 5 with *Polyporus* mycelium, and soil 4 with chitin). None of the differences in the last 4 cases is much below − 2, and when the 5 abnormal series are omitted from the total, we obtain a result which is within the range of expectation. Thus, when this medium is used for microbial counts in connection with decomposition experiments with organic matter in soil, it is necessary to reckon with the possibility that the method may break down should a flora arise for which the medium is not suitable. Especially is this the case with the actinomycetes. For the ordinary soil flora, on the other

Table VI. *Values of χ^2 in different soils. Counts on mannite-asparagine agar.*

Soil no.	Addition		Number of sets (S)	$S(n)$	Total χ^2	Difference $\sqrt{2\chi^2}-\sqrt{2n-1}$
		Soils without organic matter.				
1	Nothing	Bact.	9	51	*80·61*	*2·63*
		Act.	9	51	35·44	−1·63
5	Nothing	Bact.	13	62	59·03	−0·22
		Act.	13	62	*39·03*	*−2·25*
	Total	Bact.	—	113	139·64	1·71
		Act.	—	113	*74·47*	*−2·80*
		Soils with organic matter.				
1	Farmyard manure	Bact.	9	52	61·42	0·91
		Act.	9	52	34·23	−1·87
1	Do. + oat straw	Bact.	9	47	56·10	0·93
		Act.	9	47	36·03	−1·16
5	Mycelium of *Asp. fumigatus*	Bact.	8	37	33·26	−0·72
		Act.	8	37	41·05	0·53
5	Mycelium of *Polyporus* sp.	Bact.	9	43	39·46	−0·34
		Act.	9	43	*18·48*	*−3·15*
Series with $S(n)$ less than 30:						P
5	Mycelium of *Trichoderma*	Bact.	5	22	18·38	0·70–0·50
		Act.	5	22	22·79	0·50–0·30
5	Mycelium of *Zygorhynchus*	Bact.	6	27	24·39	0·70–0·50
		Act.	6	27	18·77	0·90–0·80
5	Mycelium of *Act. griseus*	Bact.	5	24	26·26	0·50–0·30
		Act.	5	24	25·81	0·50–0·30
5	Protoplasm of Bacterium sp.	Bact.	5	21	32·20	0·90–0·95
		Act.	5	21	21·47	0·50–0·30
						Difference
	Total	Bact.	—	273	291·47	0·79
		Act.	—	273	218·63	−2·44
Omitting the series with abnormal variation:						
	Total	Act.	—	230	200·15	−1·41

hand, the dextrose-casein agar seems well adapted to the counting of both bacteria and actinomycetes.

In Table VI the data obtained with mannite-asparagine agar from soils with and without organic matter have been treated in a similar way[1].

There is here an excessive variation in the bacterial counts in one of the soils without addition, but in all the others, as well as in the total (Table II), the variation is normal. The actinomycetes counts show subnormal variation in one of the soils without addition. Among the soils with organic matter, only one shows a definite subnormality; the others seem mostly to tend in the same direction, although the totals of χ^2 and *n* in soils with organic matter show a variation within the range of expectation, when the abnormal series is omitted. The mannite-asparagine agar thus seems under these conditions to resemble the dextrose agar, but whether it has more or less tendency to give subnormal variation than the latter medium can hardly be ascertained from the data available here.

The paper published by Waksman (8) provides sufficient data for the calculation of the distribution of χ^2 from counts of actinomycetes in soil.

Table VII. *Distribution of* χ^2 *in counts of actinomycetes (Waksman's data).*

Sets with 10 plates

χ^2	Expected	Observed
0	0·5	1
2·088	0·5	1
2·532	1·5	0
3·325	2·5	3
4·168	5	5
5·380	5	3
6·393	10	11
8·343	10	8
10·656	5	6
12·242	5	5
14·688	2·5	1
16·919	1·5	3
19·670	0·5	1
21·666	0·5	2
Total	50	50

Sets with 8 plates

χ^2	Expected	Observed
0	0·5	0
1·239	0·5	0
1·564	1·5	1
2·167	2·5	
2·833	5	4
3·822	5	2
4·671	10	7
6·346	10	7
8·383	5	4
9·803	5	1
12·017	2·5	11
14·067	1·5	1
16·622	0·5	0
18·475	0·5	8
	50	50

The medium used was dextrose-albumen agar (7), which gave slightly lower figures than casein agar. From Waksman's data 50 10-plate counts and 50 8-plate counts have been examined and the values of χ^2 calculated. The distributions are seen in Table VII.

[1] Only 78 counts from soils 1 and 5 included here; the remaining 2 were from soil 2.

In the 10-plate series there is a good agreement between observation and expectation, but in the 8-plate series there is a marked tendency to excessive variation. These latter figures are all from one particular date of sampling and the effect may therefore be due to the temporary appearance of antagonistic organisms, depressing the development of actinomycetes on some of the plates (cf. Fisher, Thornton and Mackenzie (2)).

Rao and Subramanyan (3) compared a large number of agar media for counting soil actinomycetes. They finally adopted a medium containing starch and asparagine besides mineral nutrients, because parallel counts were more consistent on this than on any other medium tested by them. In their Tables XVI and XVII, they record the mean number of actinomycetes colonies ($\bar{x}$) and the variance $\left(V = \frac{S(x-\bar{x})^2}{n}\right)$ in 13 sets of counts on starch-asparagine agar and 10 sets on soil-extract agar. This enables us to calculate the values of $\chi^2 \left(= \frac{4V}{\bar{x}}\right)$, there being 5 parallel plates in each count, for actinomycetes counts on these two media. These results are recorded in Table VIII, which shows a very strong subnormality on starch agar, but on soil-extract agar a χ^2 which varies about a normal mean value.

Table VIII. *Counts of soil actinomycetes on starch agar and soil-extract agar in parallel soil samples (Rao and Subramanyan's data).*

Sample no.	Starch-asparagine agar			Soil-extract agar		
	$\bar{x}$	V	χ^2	$\bar{x}$	V	χ^2
1	38·6	9·3	0·96	30·3	51·0	6·73
2	40·2	3·7	0·37	36·2	16·5	1·64
3	38·5	2·3	0·24	37·3	19·0	2·09
4	36·6	3·3	0·36	39·0	51·5	5·28
5	39·2	5·7	0·58	43·0	150·5	14·00
6	38·8	5·7	0·59	36·0	12·0	1·50
7	38·8	6·7	0·67	30·0	5·5	0·73
8	38·0	5·0	0·53	38·2	85·4	8·94
9	39·2	6·7	0·68	37·2	77·5	8·33
10	40·2	3·5	0·35	34·8	31·6	3·59
11	39·4	10·3	1·05	—	—	—
12	38·4	4·5	0·47	—	—	—
13	39·2	11·2	1·14	—	—	—
Total χ^2	—	—	7·99	—	—	52·83
$S(n)$			52·00			40·00
$\sqrt{2\chi^2} - \sqrt{2n-1}$		−6·15			+1·40	

In their Table XV, Rao and Subramanyan give the counts of actinomycetes colonies on individual plates (4 or 5 parallels) of starch agar from 6 different soils. A calculation of χ^2 from these data shows (Table IX) a general tendency to subnormality, and a total of χ^2 which, as shown by Fisher's Table III, is quite outside the range of expectation. The tendency to subnormal variation is thus not limited to the one particular soil mentioned in Table VIII, and the medium therefore does not seem well adapted for counting soil actinomycetes.

Table IX. *Counts of actinomycetes on starch-asparagine agar in 6 soils (Rao and Subramanyan's data).*

Soil no. ...	I	II	III	IV	V	VI
$\bar{x}$	30·5	31·4	4·8	40·2	14·5	12·6
χ^2	0·36	0·61	0·58	1·69	0·90	0·41
n	3	4	4	4	3	4

$S(n) = 22$. Total $\chi^2 = 4{\cdot}55$.

SUMMARY.

1. Eighty counts of soil bacteria and actinomycetes, made on mannite-asparagine agar and dextrose-casein agar, showed that the latter medium gave significantly higher numbers of bacteria in 76 per cent. of cases, and significantly higher numbers of actinomycetes in 60 per cent. of cases.

2. On mannite-asparagine agar the values of χ^2 of the bacterial counts were as a whole in agreement with expectation. The counts of actinomycetes colonies on this medium showed a tendency to subnormal variation.

3. 357 counts of soil bacteria and actinomycetes on dextrose-casein agar gave distributions of χ^2, which mostly agreed with expectation. The bacterial counts showed a tendency to excessive, the actinomycetes counts to subnormal variation. These abnormalities seemed most liable to occur in soils where special microfloras had been accumulated as a result of the introduction of decomposable organic matter.

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CCXI. THE LIBERATION OF ELEMENTARY NITROGEN BY BACTERIA.

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THE loss of nitrogen during the putrefaction of organic matter has been the subject of numerous investigations during the past 100 years. In a review of the literature of the subject by Jensen [1906] there appears to be a difference of opinion as to the source of the nitrogen loss and of the conditions which give rise to it. Thus Reiset [1856], Dehérain and Dupont [1898] and Gibson [1895] found that nitrogen was a definite end-product in the putrefaction of meat. Lawes, Gilbert and Pugh [1861] also found that nitrogen was evolved in the aerobic decomposition of vegetable matter, and Schloesing [1889, 1] tried to confirm this result but not very successfully.

This conclusion was not supported by the work of Hüfner [1876], Kellner and Yoshi [1888], Tacke [1887] and Immendorff [1892]. These authors found that the liberation of free nitrogen only occurred in the presence of nitrites or nitrates. Jensen is of opinion that the results of the earlier workers are not reliable owing to faulty analytical methods.

Löhnis [1926] states that a loss of nitrogen from organic matter occurs only in the presence of air, and that it has not yet been proved that free nitrogen is derived from the amino-group or ammonia. He suggests the possibility of a liberation of nitrogen by the purely chemical interaction between amines or NH_3 and nitrous acid thus:

$$\begin{aligned} R.NH_2 + HNO_2 &= R.OH + N_2 + H_2O \\ NH_3 \quad + HNO_2 &= N_2 + 2H_2O \end{aligned}$$

This possibility was first put forward by Dietzell [1882], though the reaction was first discovered by Piria [1849]. Fowler and Kotwal [1924] found it to be ineffective except in the presence of mineral acid. Grimbert [1899], however, suggested that a liberation of amino-nitrogen may occur in a nitrate-broth medium without the development of acidity or perhaps of only local and temporary acidity.

Russell [1927] agrees with Jensen as to the unreliability of the analytical methods used by the earlier workers but considers the results of Lawes, Gilbert and Pugh [1861] and of Pfeiffer *et al.* [1897] as sufficient evidence of the possibility of nitrogen being a final product of protein breakdown. In collaboration with Richards [1917], however, he showed that no nitrogen was evolved in the decomposition of dung under completely aerobic or completely anaerobic conditions but only under a combination of the two conditions. He accounted for the result of Lawes, Gilbert and Pugh and others by the formation under anaerobic conditions of unstable intermediate products which evolve nitrogen on contact with air. Waksman [1927] in his review of the literature does not refer to the possibility of the liberation of free nitrogen without denitrification.

Since the work of Lawes, Gilbert and Pugh, and of Pfeiffer on the liberation of nitrogen from organic matter by oxidation, three authors have recorded the same phenomenon under anaerobic conditions. Wood and Willcox [1897] obtained considerable quantities (150 cc.) of nitrogen from a peptone-glucose medium inoculated with a pure culture of a bacillus isolated from fermenting bran used in the bating of hides. Unfortunately this experiment is not above criticism since oxygen gas was also collected, which, as a product of anaerobic fermentation, suggests the leakage of air into the apparatus. Harden [1901], using a peptone-glucose medium inoculated with *B. coli*, obtained a small amount of nitrogen (7·5 cc.) but none in absence of glucose. The rôle of the sugar in promoting the evolution of nitrogen is somewhat obscure, since carbohydrates are known to have a depressing effect on ammonification [Doryland, 1916], which would presumably be an intermediate stage in the formation of free nitrogen. Bach and Sierp [1923], using sewage sludge, obtained considerable quantities of nitrogen during anaerobic (?) fermentation in the presence of large volumes of tap-water. As pointed out by Buswell and Neave [1927] the results are subject to a very large error from the daily exchange of 500 cc. of tap-water containing sufficient dissolved air to account for all the nitrogen collected.

Thus, the evidence amply bears out Pfeiffer's remarks that "there is scarcely another field of research in agricultural chemistry in which we encounter contradictions so numerous and so fully unexplained."

As Jensen points out, the position regarding the formation of free nitrogen by bacteria is complicated, not only as regards the source of the nitrogen but also as to the conditions which give rise to it.

Amidst the conflicting evidence of previous workers there is one process of the liberation of free nitrogen by bacteria that is beyond question, *viz.* denitrification. According to Mair [1908], Beijerinck and Minkman [1910] and Lloyd and Cranston [1930], nitrates are reduced in three stages: (1) nitrite, (2) hyponitrite, (3) free nitrogen. It follows therefore that in all attempts to prove the liberation of nitrogen by the oxidation of amines or ammonia it is essential to ensure the absence of nitrates, nitrites and hyponitrites. The presence of nitrifying bacteria during putrefaction has been well established by numerous workers. These organisms are now recognised as taking part in biological oxidation in symbiosis with heterotrophic organisms [Mair, 1908; Barritt, 1931].

Ammonification studies have been carried out by numerous workers in recent years, and the production of ammonia is now regarded as an essential part of the bacterial oxidation of organic matter when the C/N ratio of the substrate is not too high [Marschal, 1893; Doryland, 1916]. The yield of ammonia is quantitative and no recent worker has recorded a loss of free nitrogen. The only certain products of the biological oxidation of ammonia are nitrite and nitrate, and, since a crude inoculation of soil organisms will oxidise ammonia quantitatively to nitrous or nitric acid [Schloesing, 1889, 2], there is no reason to suppose the existence in soil of organisms capable of oxidising ammonia to free nitrogen.

These observations are amply borne out by the work of Russell and Richards [1917] on the loss of nitrogen during the decomposition of manure, and by the work of the Royal Commission on Sewage Disposal [1908]. In both cases loss of nitrogen was only recorded in the presence of nitrates, which represent the balance of the nitrification process in excess of denitrification as stated above.

Since denitrification appears to be an essential part of the process, the following investigations were undertaken with a view to obtaining more precise data on this process and also on the possible interaction of amines and ammonia with nitrous acid, a subject which has received little attention from previous workers. The experiments of Fowler and Kotwal [1924] on this point involved small gasometric measurements (2 cc.) and are not at all convincing.

EXPERIMENTAL.

Culture solutions of 0·5 % concentration of the following substances were prepared with the addition of 0·05 % potassium phosphate, 0·03 % sodium chloride and 0·1 % potassium nitrate: urea, uric acid, glycine, alanine, tyrosine, ammonium acetate, ammonium sulphate, dextrose + ammonium sulphate, dextrose, acetic acid and alcohol. The distilled water used was previously inoculated by shaking each litre with 1 g. of Broadbalk soil. The initial p_H was adjusted to 6·5. 200 cc. of each culture were incubated at 25° in a 300 cc. stoppered bottle for 6 weeks. Determinations of organic *plus* ammonia nitrogen were made by the Kjeldahl method, nitrites by the Griess-Ilosvay method and nitrate by the pyrogallol colorimetric method.

During the incubation period the reaction of the culture solutions was tested daily. The rise in p_H due to ammonification of organic nitrogen and liberation of alkali by the denitrification of the potassium nitrite was corrected by the addition of solid KH_2PO_4, and the lowering of p_H due to glycolysis was corrected to some extent by the addition of calcium carbonate. In this way an average p_H of 6·5 was maintained.

The formation of nitrite occurred in all cultures within 24 hours and persisted until all the nitrate had disappeared. The amount of nitrite produced varied with the different solutions, the greatest amounts (0·013 %) being recorded in the solutions containing dextrose and alcohol. The nitrite also persisted in these solutions throughout the whole period of 6 weeks. The most vigorous reduction occurred in the solutions of uric acid and amino-acids, in which cases the amount of nitrite never exceeded 0·003 % and completely disappeared within 24 days. The slower rate of reduction in the carbohydrate solution is probably due to the lower p_H owing to the buffering action of the calcium carbonate being arrested by a covering of bacterial growth.

The formation of organic acids from dextrose does not require oxygen and the oxidation of these acids is very much reduced at $p_H < 6$ [Cook and Alcock, 1931]. For this reason the nitrite is more slowly reduced and therefore persists in the carbohydrate solutions for a much longer period than in the solutions containing amino-compounds. The results of the nitrogen estimations are given in Table I.

Table I.

		Initial N g. per 100 cc.		Final N after incubation			Loss of N	
Substance	C/N ratio	organic $N+NH_3$	as N_2O_5	organic $N+NH_3$	N_2O_3	as N_2O_5	organic $N+NH_3$	as N_2O_5
1 Ammonium sulphate	—	0·053	0·014	0·0531	Trace	0·014	Nil	Nil
2 Urea	0·43	0·233	0·014	0·235	Trace	0·014	Nil	Nil
3 Uric acid	1·07	0·167	0·014	0·166	—	—	0·001	0·014
4 Glycine	1·71	0·0933	0·014	0·0928	—	—	0·0005	0·014
5 Ammonium acetate	1·71	0·0530	0·014	0·0523	—	—	0·0007	0·014
6 Alanine	2·57	0·0777	0·014	0·0776	—	—	0·0001	0·014
7 Tyrosine	7·7	0·0379	0·014	0·0372	—	—	0·0007	0·014
8 Dextrose + $(NH_4)_2SO_4$	10·0	0·0127	0·014	0·0124	—	0·0136	0·0003	0·0004
9 Dextrose only	14·5	—	0·014	0·00501	—	—	(increase 0·00501)	0·009
10 Acetic acid	14·5	—	0·014	0·00093	0·004	0·009	(increase 0·00093)	0·0007
11 Alcohol	18·0	—	0·014	0·00125	—	—	(increase 0·00125)	0·0127
12 Glycine + dextrose	3·5	0·0933	0·014	0·0916	0·0025	—	(loss 0·0017)	0·0115

It will be seen that there is no significant loss of organic nitrogen or ammonia in any of the solutions except No. 12 (glycine + dextrose), in which case a loss of 0·0017 g. nitrogen occurs. Comparing this result with that of No. 4 (glycine alone) it appears that the addition of dextrose to culture No. 4 (glycine alone) has resulted in a loss of organic nitrogen.

In the case of cultures Nos. 1 and 2, ammonium sulphate and urea, there was also no loss of nitrate nitrogen. Ammonification of the urea occurred, but no further change.

In cultures Nos. 3, 4, 5, 6 and 7 (of low C/N ratio) denitrification was complete in 21 days, and although these were incubated for a further period of 21 days no further loss of nitrogen occurred. Therefore the organisms concerned in the liberation of nitrogen during the first 3 weeks were unable to liberate nitrogen from either ammonia or organic nitrogen.

The higher C/N ratios of cultures Nos. 9, 10 and 11 appear to be associated with a smaller loss of nitrate nitrogen and a slight increase in organic nitrogen due to synthesis.

The loss of nitrogen in culture No. 12 appears at first sight to confirm Harden's observation of the liberation of nitrogen from protein in the presence of sugar. In the above case however nitrites were present, in which respect it differs from Harden's unless nitrates or nitrites were present as an impurity. Considerable amounts of lactic acid were formed in Harden's culture with glucose and it is well known that nitrites may react with amino-acids in presence of organic acid (*e.g.* acetic acid in Van Slyke's method of estimating amino-N). We may therefore expect a loss of amino-nitrogen in the presence of organic acids and nitrite. If nitrite was present in Harden's culture, free nitrogen should also have been formed in the peptone solution without glucose by the denitrification process. In this case however the amount of nitrogen liberated would be exactly half that formed by the interaction of amines with nitrous acid. Possibly 3·5 cc. of nitrogen is not sufficient for measurement with any degree of accuracy [see Russell and Richards, 1917]. In the cases where nitrite is present at the end of the fermentation the recorded loss of organic nitrogen might have occurred by means of the interaction between amines and nitrous acid on adding sulphuric acid for the Kjeldahl digestion. The possible loss of nitrogen at this stage was tested by adding excess nitrite to a 0·5 % solution of glycine before the acid digestion. The final organic nitrogen was identical with that of the materials without the glycine, indicating complete decomposition of the amino-group. In culture No. 12 therefore the recorded loss of organic nitrogen may have occurred during analysis and not during the fermentation.

In order to test the possibility of this reaction occurring during acid fermentation sterile solutions of glycine and sodium nitrite were prepared and varying quantities of lactic acid added. The amino-nitrogen was determined by Sørensen's method of formaldehyde titration. The results obtained are given in Table II.

Table II.

			Initial p_H	cc. $N/10$ NaOH required
0·5 % glycine	+ 0·5 % $NaNO_2$		6·5	31·0
,,	,,	+ lactic acid	6·2	30·6 (12 hrs.)
,,	,,	,,	5·7	30·5 ,,
,,	,,	,,	4·8	30·5 ,,
,,	,,	,,	4·2	28·4 ,,
,,	,,	,,	3·8	25·8 (5 mins.) 7·8 (12 hrs.)

These results show that the rate of decomposition of the amino-acid is considerably diminished above p_H 4. It was afterwards found that the p_H of the less acid solutions increased rapidly, so that an initial p_H of 4·2 became 5·2 in 10 minutes and 6·5 in 1 hour. This rise in p_H is probably due to the volatility of the nitrous acid. At $p_H < 5·0$ a rapid evolution of gas occurred only on shaking the flask, and no gas appeared to be evolved when the solution remained quiescent. This liability to supersaturation with nitrogen gas must invalidate the results obtained by gasometric methods unless accompanied by vigorous fermentation. With a continuous production of organic acid such as occurs in the fermentation of carbohydrates a more effective interaction between the amino-acid and nitrites could occur and result in a greater loss of free nitrogen.

Under strongly aerobic conditions organic acids are oxidised to carbonic acid with a consequent rise in p_H of the medium but, since nitrous acid is also liberated from nitrites by carbon dioxide [Moore, 1904], it seemed desirable to repeat these experiments with carbonic acid in place of lactic acid. Solutions of carbonic acid were obtained from a sparklet-siphon, using distilled water and liquid CO_2. A solution of calcium bicarbonate was also used. The results obtained are as follows:

Formaldehyde titrations of 0·5 % glycine + 0·5 % nitrite

	p_H	cc. $N/10$ NaOH required
In water only	6·8	31·0
In supersaturated solutions of CO_2 at atmospheric pressure 12 hours	5·8	23·2
In saturated solution of CO_2 under pressure	5·8	20·5
In calcium bicarbonate solution	6·0–6·8	30·0

These results show that it is possible to obtain a loss of nitrogen by the interaction of glycine and nitrite in a solution supersaturated with carbon dioxide. It is unlikely that such conditions would occur in the decomposition of organic matter. In the culture solutions described above (Table I) the reaction was maintained at p_H 6·5 and no loss of amino-nitrogen occurred [*cf.* Grimbert, 1899].

From these results it must be concluded that a loss of organic nitrogen is possible during denitrification provided that organic acids and amino-compounds are simultaneously produced. This event is more likely to occur when readily available carbohydrates like dextrose are present. It would also be expected to occur in the absence of nitrates when anaerobic conditions are followed by aeration. In the presence of nitrifying organisms amino-acids may be regarded as the unstable compounds referred to by Russell and Richards [1917] as oxidising to elementary nitrogen on exposure to air.

The simultaneous production of ammonia by the oxidation of protein and of nitrite by the reduction of nitrate suggests the possibility of free nitrogen being formed by the spontaneous decomposition of ammonium nitrite. This has been suggested by both Löhnis and Waksman. It is well known that ammonium nitrite is readily decomposed on heating, but in dilute solution it appears to be quite stable. A 0·5 % solution accurately prepared by mixing molecular equivalents of ammonium sulphate and sodium nitrite was incubated at 25° for 30 days without loss of either ammonia or nitrite and without a trace of gas being produced. The same result was obtained when the solution was incubated with soil. It must therefore be concluded that the decomposition of ammonium nitrite is not a source of the liberation of free nitrogen by bacteria.

SUMMARY.

1. A review of the literature shows that much confusion exists regarding the liberation of nitrogen by bacteria. The evidence of some workers that it occurs under anaerobic conditions is cancelled by that of others who show that it occurs only under aerobic conditions.

2. It is pointed out that inaccuracies in analytical methods and insufficient attention to the occurrence of nitrates or nitrification is responsible for the confusion as to the nature of the process.

3. Experiments are described which confirm the results of several previous workers that

(*a*) free nitrogen is liberated by soil bacteria during denitrification;

(*b*) there is no evolution of nitrogen from organic compounds under anaerobic conditions in the absence of oxidised nitrogen, nor from oxidised nitrogen in the absence of oxidisable organic compounds.

4. The reduction of nitrates and nitrites is effected most rapidly in the presence of compounds of low C/N ratio, and the formation of free nitrogen is strictly confined to the reduction of oxidised nitrogen provided the reaction of the medium does not fall below p_H 6·5.

5. The production of free nitrogen from organic compounds does not occur by bacterial action but only indirectly by the interaction between free nitrous acid and amino-compounds, both of which may be produced simultaneously by bacterial agency.

6. Free nitrous acid is liberated from nitrites by organic acids and carbonic acid, but does not effect the decomposition of amino-acids unless the p_H of the medium is $< 6·0$.

7. The presence in a culture of readily available carbohydrates may result in a loss of free nitrogen from organic matter by the indirect process referred to in 5.

8. In Kjeldahl determinations of solutions of organic nitrogen the presence of nitrite results in a loss of amino-nitrogen during the acid digestion.

9. There is no liberation of free nitrogen by the decomposition of ammonium nitrite in culture solutions at ordinary temperatures.

The writer is much indebted to Mr R. G. Warren of the Chemical Department, for much useful advice and for the control of the Kjeldahl determinations, and also to Dr L. de Telegdy Kovato of Budapest (late of this department) for help in the discussion of the literature.

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THE GROWTH AND RESPIRATION OF BACTERIA IN SAND CULTURES IN THE PRESENCE AND ABSENCE OF PROTOZOA[1]

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(With 8 Text-figures.)

PART I.

The results of Cutler, Crump and Sandon (1) and of the author in Hungary (14), on the counting of protozoa and bacteria in the soil have shown that an inverse correlation exists between their numbers. This result has led to the conclusion that protozoa have the effect of reducing the number of bacteria by their phagocytic action not only under the special circumstances described by Russell and Hutchinson (10, 11), but also under ordinary field conditions.

On the other hand very little information is available as to the other activities of protozoa in soil, though more recent work has given some very unexpected results. Nasir's (9) experiments showed that the presence of protozoa in artificial culture media or in sand cultures had no depressing effect on nitrogen fixation, but that on the contrary it caused a great increase in the amount of nitrogen fixed. In the year 1926 the same result was confirmed in different parts of the world. Cutler and Bal (2) in England suggested that the increased nitrogen fixation might be due to the efficiency of *Azotobacter* being maintained for a longer period; Keizo Hirai and Iwao Hino in Japan (7) also found that nitrogen fixation was generally stimulated in the presence of protozoa. In their opinion the soil protozoa and bacteria live in a state of disjunctive symbiosis, in other words the presence of soil protozoa decreases the acidity of the nutrient medium, which results in a vigorous growth and increased nitrogen fixation. The present writer in Hungary (13), at the same time and without previous knowledge of these results, published the results of an investigation on the influence of various artificial zeolites on nitrogen fixation by pure and mixed *Azotobacter* cultures. It was found that the nitrogen-fixing power of the new cultures, especially those infected by protozoa (*Paramoecium* sp. and *Colpidium* sp.) in the presence of readily available salts, was greater than that of pure cultures; this effect being probably due to the stimulating effect of the protozoa. In 1928 Fedorowa-Winogradowa (4) carried out experiments cultivating *Azotobacter* and soil amoebae together on sterile soil and stated that, while the amoebae reduced the number of *Azotobacter*, at the same time they stimulated their rate of reproduction. In sterile medium consisting of 1 per cent. mannitol solution and soil the development of *Azotobacter* was more vigorous in the presence than in the absence of amoebae.

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[1] This work was carried out at Rothamsted whilst the writer held a post-graduate Fellowship of the Royal Hungarian Ministry of Education.

The protozoa have also a decidedly beneficial effect on other common soil processes. Hills (6) studied the accumulation of available nitrogen (NH_3 and NO_3) in sterilised soil; on the one hand reinoculated with soil known to contain protozoa, and on the other with a crude culture of bacteria obtained by picking several colonies from agar plates which had been poured from soil dilutions. He obtained no evidence of decreased accumulation of available nitrogen in the presence of protozoa. Waksman (15) found that the protozoa seemed to have a detrimental effect upon the numbers of bacteria but not upon their ammonifying efficiency. Skinner (12) partially sterilised soil, and after reinoculating with various cultures he found that *Hartmanella hyalina* caused a reduction in the number of bacteria and a slight depression in carbon dioxide evolution and ammonia accumulation; the fungi *Trichoderma Köningi* and *Penicillium*, however, caused an increase in carbon dioxide evolution and a decrease in ammonia accumulation. Neither of these workers attempted to follow the course of ammonia formation by consecutive observations. Meiklejohn (8) carried out two sets of experiments on ammonia production from peptone, one with bacteria in liquid cultures and another in which bacteria and *Hartmanella* were compared with bacteria alone in sand cultures. The presence of amoebae, while lowering the bacterial numbers, appeared to increase the rate of ammonia production, and it is suggested that the amoebae reduced the bacterial numbers from too high a value to a value nearer the optimum for ammonia production, and so increased the rate of ammonia production.

With regard to the carbon dioxide production from soil it is well known that this is probably a better index of soil fertility than the actual number of soil bacteria; though many investigators have found a close connection between carbon dioxide evolution, numbers of soil bacteria and soil fertility. According to the experiments of Cutler and Crump (3) on carbon dioxide evolution from soil and sand cultures containing a species of bacterium with and without amoebae, however, the correlation would appear to be not such a simple one as that inferred from the previous work, since bacterial numbers and carbon dioxide production are correlated only provided that the amoebae are not present, or are present in very small numbers. In sands containing peptone, the amoebae caused a decrease in carbon dioxide production, but in sands containing mineral salt solution and glucose or soil extract the reverse effect was obtained.

From the above results it seems to be well established that soil protozoa may not only act as harmful factors in the life of soil bacteria, but they may also stimulate bacterial development, resulting in further biological transformations in soil. This fact may be connected with their phagocytic action, but up to the present little work has been done in this direction. It was felt that the importance of this problem in soil processes was such as to warrant further investigations on the influence of protozoa on carbon dioxide production. The problem involves so many factors and is one of such great complexity, that it was necessary to simplify it. This was effected by studying the influence of protozoa (especially Ciliates and Flagellates) on the carbon dioxide production of mixed and pure bacteria cultures in sand containing definite compounds.

METHODS.

Although soil would undoubtedly appear to be the best medium to use, preliminary experiments showed that the results were difficult of interpretation owing to its complex nature. The problem was therefore simplified by using sand. Silver sand, which passed a 1 mm. sieve, was digested with strong hydrochloric acid for 24 hours, washed free of acid in running water, dried and ignited. Four hundred gm. portions of this were then placed in 2 litre Erlenmeyer flasks and sterilised in an autoclave under 15 lb. pressure for 30 min. The moisture contents were made up by adding the following two nutrient solutions: (1) mineral salt solution + 0·5 per cent. peptone; (2) mineral salt solution + ammonium sulphate + 0·6 per cent. glucose (ratio C/N = 3·5 : 1) in amount equivalent to 16 per cent. of the weight of the dry sand.

The method of inoculation, if not stated otherwise, was as follows. Four different cultures were employed: (*a*) protozoa[1] + bacteria from Barnfield farmyard manure plot; (*b*) mixed soil bacteria from soil dilutions enriched by several sub-cultures in peptone, or ammonium sulphate media; (*c*) pure cultures of "YB" bacteria either without *Colpidium* sp. or (*d*) with the addition of *Colpidium* sp. The sand after thoroughly mixing with the nutrient media was inoculated under aseptic conditions by spraying it with 10 cm. of the cultures. On account of the sticky nature of the peptone the sand was stirred every day throughout the experiment. This was done aseptically for both series of cultures. Bacteria and protozoa were counted daily by the dilution method used in this laboratory. Later, in order to facilitate the work, only bacterial counts were made daily, but microscopic examination of a small quantity of sand in a drop of sterile water on the slide was carried out every day to ensure that the protozoa were growing satisfactorily.

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[1] The protozoa consisted of *Oicomonas termo*, *Cercomonas crassicauda*, *Heteromita* sp. and other Flagellates.

For the determination of the carbon dioxide production the Pettenkoffer method was employed, using 0·2 per cent. baryta solution for the absorption and $N/5$ hydrochloric acid for titration with phenolphthalein. In every case the cultures were aerated by drawing carbon dioxide free air over them 10 hours daily by means of an aspirator. Before each experiment was begun the apparatus was made free of carbon dioxide by ½ hour aeration with carbon dioxide free air. The baryta solution was titrated at least once in every 24 hours or oftener if necessary. All the experiments were repeated three or four times under quite uniform conditions.

RESULTS.

In the first experiments with peptone the carbon dioxide evolution began slowly and reached its maximum in the second or third day in the majority of cases and then fell, first sharply and later more steadily. During this time the growth of bacteria followed quite closely the evolution of the carbon dioxide, and as found by Cutler and Crump (3), it reached its maximum a day later. This occurred in all the experiments made on peptone media inoculated either with bacteria only or with bacteria and protozoa. No explanation for this interesting observation has yet been found. In other respects, however, there were great differences according to the inoculations, since the cultures containing protozoa produced always much larger amounts of carbon dioxide than those with bacteria alone, and the mixed soil bacteria also produced more carbon dioxide than the pure "YB" culture. Typical curves showing the carbon dioxide production during the experiments and the differences between the amounts given off in various cultures are given in Fig. 1, calculated in mg. carbon dioxide per gm. of sand.

Another picture is obtained in the experiments with ammonium sulphate and glucose. In this case a C/N ratio of 3·5 was chosen in order to correspond with the C/N ratio of the peptone solution. In these experiments the connection between the bacterial growth and carbon dioxide production was not so close as before, though a similar lag of the maximum bacterial number behind the maximum of carbon dioxide production could be observed in the majority of cases. This experiment was characterised by considerable fluctuations in bacterial numbers

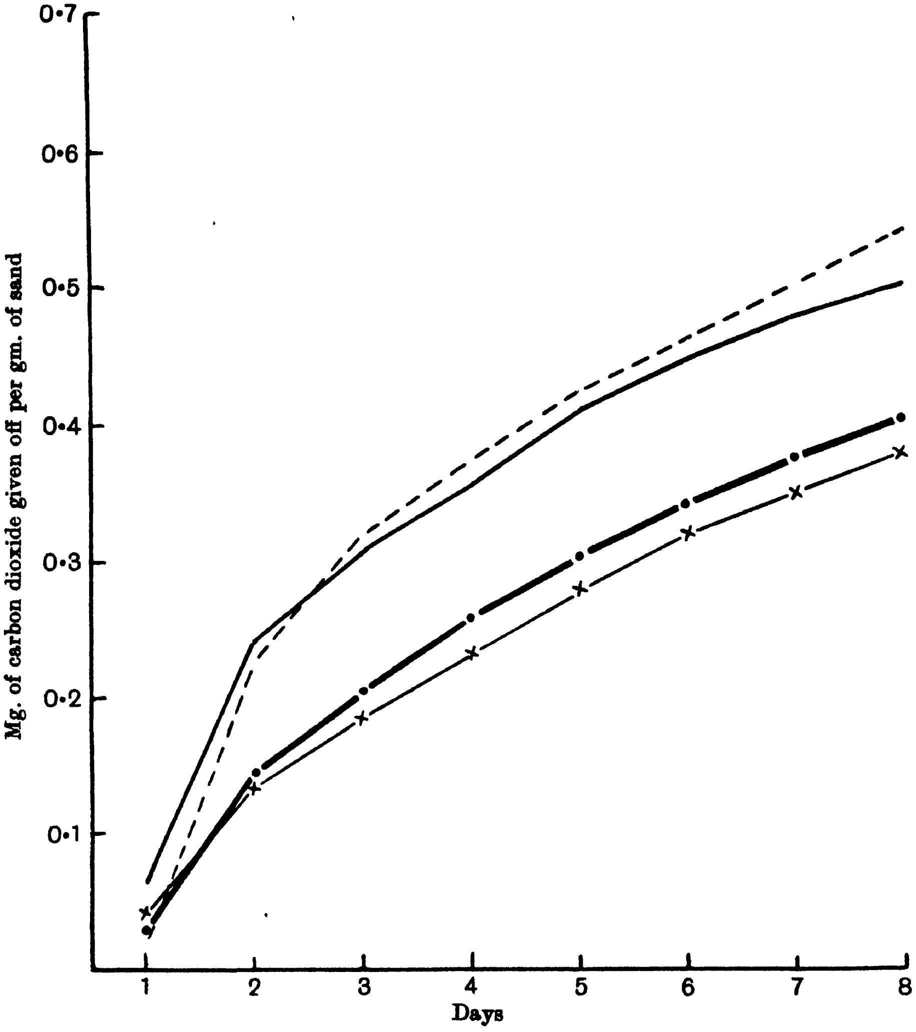

Fig. 1. Influence of protozoa on the CO_2 production. Peptone.
- - - - - Soil bacteria and protozoa, mixed culture.
——— "YB" bacteria and *Colpidium* sp.
●——● Mixed soil bacteria.
×——× "YB" bacteria.

which contrasted with the uniformity observed in the peptone experiment.

To study the question whether a close relation existed between the number of bacteria and carbon dioxide produced, contingency tables were drawn up in which the two variables were the carbon dioxide production and the numbers of bacteria. An increase in either variant is shown by a + sign and a decrease by a − sign, if both increase or decrease together it will be shown by + + or − −, if they vary inversely by + − or − +. If there are a sufficient number of cases, and the variables are wholly independent, there will be equality between the like and unlike signs; a preponderance of like or unlike signs will show that the two variables are related one to another. To test the significance of any departure from equality a χ^2 was worked out: if the χ^2 is greater than 4 it may be assumed that there exists a relation between the variables which is not due to chance.

In Table I it is shown that the relationship was very close between the bacterial numbers and carbon dioxide produced in the peptone experiments, not only when bacteria were present alone, but also in the presence of protozoa. In the medium containing glucose + ammonium sulphate the value of the test of relationship is hardly significant, especially in the presence of protozoa. It is interesting to note that the number of bacteria on peptone media often reached several thousand millions, while in the glucose + ammonium sulphate media greater numbers than 400 millions per gm. were not observed. In view of the lower bacterial numbers on the glucose medium it was thought desirable to determine to what extent the carbon dioxide production could be accounted for by the fungal growth or the presence of bacteria not estimated by Thornton's medium.

Table I.

Contingency tables for carbon dioxide production and bacterial numbers in sand (CO_2 *given first*).

Sands		+ +	+ −	− +	− −	χ^2
Peptone	Bacteria alone	11	0	4	17	19·00
	Bacteria and protozoa	8	0	4	20	17·77
$(NH_4)_2SO_4$	Bacteria alone	9	2	4	9	5·97
	Bacteria and protozoa	10	4	3	7	4·03

With this object in view plate counts were made on Czapek agar for fungi and on glucose + ammonium sulphate agar for bacteria. It was found that there was no significant difference between the two sets of estimations. It must therefore be concluded that no special bacteria or fungi were responsible for the carbon dioxide production obtained.

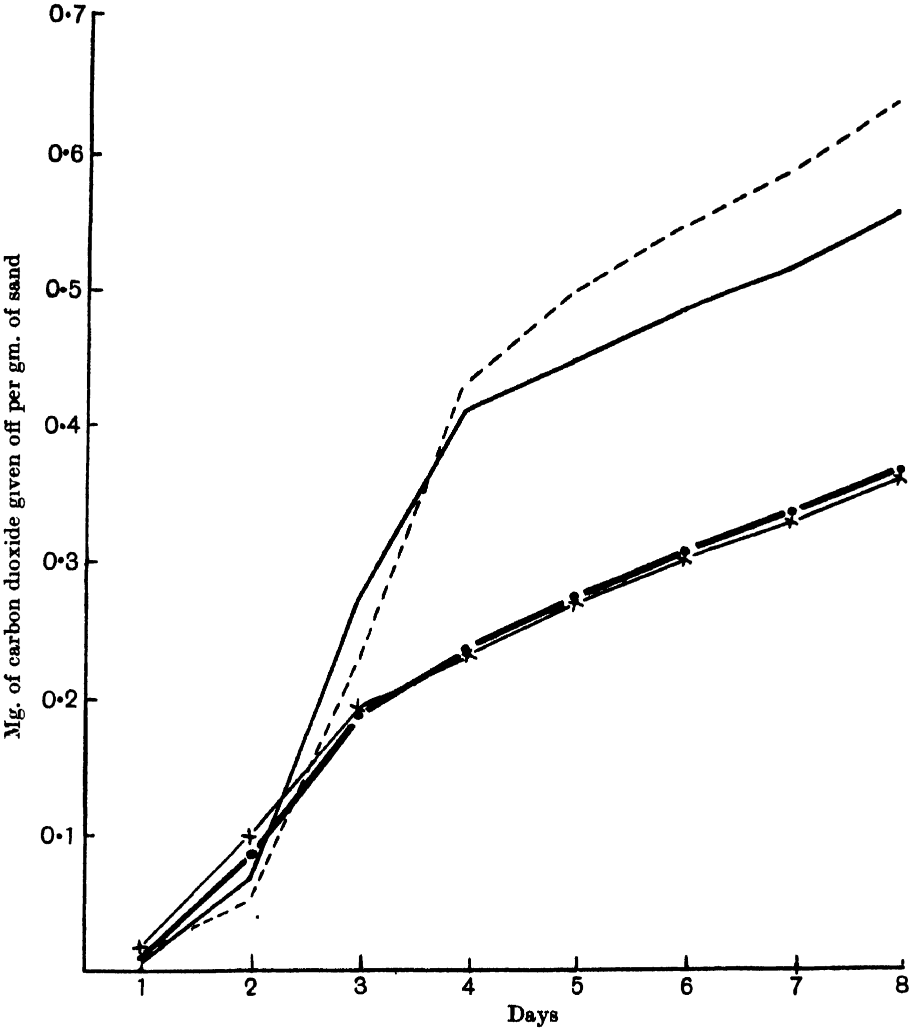

Fig. 2. Influence of protozoa on the CO_2 production. $(NH_4)_2SO_4$ + glucose.

- - - - - Soil bacteria and protozoa, mixed culture.

——— "YB" bacteria and *Colpidium* sp.

●——● Mixed soil bacteria.

×——× "YB" bacteria.

The results of the experiments made on glucose + ammonium sulphate media are shown in Fig. 2. It is interesting to note that the protozoa-free cultures gave off nearly the same amount of carbon dioxide. The cultures infected with protozoa showed an increase in the carbon dioxide evolution and this increase was especially great in the case of the culture obtained from soil and containing bacteria and protozoa.

The bacterial efficiencies are given in Table II according to the number of bacteria present per gm. of soil. For the measurement of this efficiency the amount of carbon dioxide produced during each 24-hour period was calculated per 1000 million bacteria.

Table II.

Bacterial efficiencies in gm. per 1000 *million bacteria.*

Sands	No. of cases	0–200 millions	No. of cases	200–400 millions	No. of cases	400–600 millions	No. of cases	600–800 millions	No. of cases	Over 800 millions
Peptone										
Bacteria alone	5	0·000612	5	0·000155	3	0·000083	2	0·000149	6	0·000082
Peptone										
Bacteria and protozoa	6	0·000396	6	0·000240	4	0·000277	2	0·000123	2	0·000144
$(NH_4)_2SO_4$										
Bacteria alone	12	0·000565	9	0·000249	—	—	—	—	—	—
Bacteria and protozoa	15	0·000691	6	0·000524	—	—	—	—	—	—

It is obvious from this table that the bacteria alone on both media show very varying efficiency. In the presence of protozoa, however, the differences between the efficiencies of various bacterial populations were not so large. This fact is of interest, because it indicates that the protozoa exert a control on the bacteria, or in other words they have an equalising effect on the work done by the bacteria. The bacterial numbers in pure cultures were greater in every case than in the cultures with protozoa, especially at the time of maximum carbon dioxide production; but nevertheless not only was the efficiency more uniform, but the production of carbon dioxide was also higher in the latter cases; as is shown in Table III.

Table III.

Amount of CO_2 *in gm. given off from* 400 *gm. of medium by varying numbers of bacteria.*

Sands	No. of cases	0–200 millions	No. of cases	200–400 millions	No. of cases	400–600 millions	No. of cases	600–800 millions	No. of cases	Over 800 millions
Peptone										
Bacteria alone	5	0·0103	5	0·0139	3	0·0174	2	0·0412	6	0·0333
Bacteria and protozoa	6	0·0144	6	0·0291	4	0·0539	2	0·0333	2	0·0501
$(NH_4)_2SO_4$										
Bacteria alone	12	0·0111	9	0·0298	—	—	—	—	—	—
Bacteria and protozoa	15	0·0215	6	0·0585	—	—	—	—	—	—

According to these data the effect of the protozoa is to reduce the bacterial numbers and at the same time to maintain their efficiency on the same level. In this connection it should be observed that Cutler and Crump (3) also found that the bacteria produce more carbon dioxide when their numbers are not rising and less as their numbers increase. The corresponding data obtained in the present investigation are given in the contingency Table IV, in which the two variables are the bacterial numbers and their efficiency.

Table IV.

Contingency table for numbers of bacteria and efficiency in producing carbon dioxide (efficiency given first).

Whole period

Sand	+ +	+ −	− +	− −	χ^2
Peptone	6	23	22	5	20·6
$(NH_4)_2SO_4$	8	14	16	4	8·1
Both media	14	37	38	9	28·1

It is seen that in most cases increasing efficiency is connected with decreasing bacterial numbers, the converse also being true. The significance of this fact becomes more obvious if only the last 5 days are taken in consideration (Table V).

Table V.

Contingency table for numbers of bacteria and efficiency in producing carbon dioxide.

Last five days

Sand	+ +	+ −	− +	− −	χ^2
Peptone	2	23	10	5	15·4
$(NH_4)_2SO_4$	4	14	9	3	8·1
Both media	6	37	19	8	22·9

Naturally in young cultures without protozoa where the bacterial numbers are low there is a greater production of carbon dioxide, since at this age the rapid reproduction requires a large consumption of energy which involves a rapid evolution of carbon dioxide; whereas in the presence of protozoa reproduction continues even in older cultures, resulting in a correspondingly greater production of carbon dioxide to meet the energy requirements. The rôle played by the protozoa is to reduce the number of bacteria from the beginning, and this results in a more uniform efficiency throughout the whole period; and a greater total amount of carbon dioxide is produced by smaller numbers in the same time.

From Table III it would appear that the different media are unable to support the same sized population: peptone supports a larger population than glucose and ammonium sulphate, yet in the latter medium the bacteria produce roughly double the amount of carbon dioxide, showing that it is not the greatest numbers that are able to produce the greatest amount of carbon dioxide.

The question arises whether, if the protozoa have a stimulating effect on carbon dioxide production by bacteria, there is any limit beyond which an opposite effect takes place. In other words, will a considerable increase in the protozoan population be followed by an increased carbon dioxide production or not? The study of such a question has experimental difficulties, but some preliminary investigations in this direction have been carried out. Experiments were made on peptone sand media with double inoculations, soil bacteria + protozoa as before followed by a second inoculation from a biological filter containing a rich culture of protozoa, especially flagellates, at different intervals, viz. (1) immediately, (2) after 2 days, *i.e.* during the maximum carbon dioxide production and (3) after 4 days. The results obtained are shown in Fig. 3 in comparison with an experiment without double inoculation. It is interesting that though in the double inoculation experiment new bacteria were introduced with the protozoa, the carbon dioxide production was decreased in every case. This decrease is in the first case less significant and agrees entirely with the result of another experiment where YB bacteria and *Colpidium* sp. were inoculated a second time. In the second and third case the repeated inoculation has not a stimulating effect, the carbon dioxide production is decreased and the final number of protozoa is always higher than that found in other experiments, as is shown in Table VI.

Table VI.

Final number of protozoa.

Number of protozoa per gm. of sand				
Total	Cysts	Active	Inoculation	Treatment
358,600	196,800	162,600	Protozoa and bacteria mixed culture	Sand + peptone mineral solution
421,400	236,200	185,200		
940,000	21,584	918,416	As above + protozoa and bacteria from a biological filter: (*a*) immediately	
1,310,000	21,467	1,107,933	(*b*) after 2 days	
1,880,000	25,061	1,674,939	(*c*) after 4 days	
12,600	—	12,600	"YB" bacteria and *Colpidium* sp.	
18,200	—	18,200		
486,400	211,200	275,200	Protozoa and bacteria mixed culture	Sand + glucose $(NH_4)_2SO_4$ mineral solution
502,600	216,600	286,000		
18,900	—	18,900	"YB" bacteria and *Colpidium* sp.	

The results of these investigations are summarised in Table VII, from which interesting comparisons may more easily be made. The maximum amount of producible carbon dioxide calculated on the carbon content of the nutrient media was taken as 100, so that the amounts of carbon dioxide actually produced could be expressed in percentages. In such a way a rough measurement is obtained for the differences in the carbon dioxide production of pure cultures and cultures with protozoa.

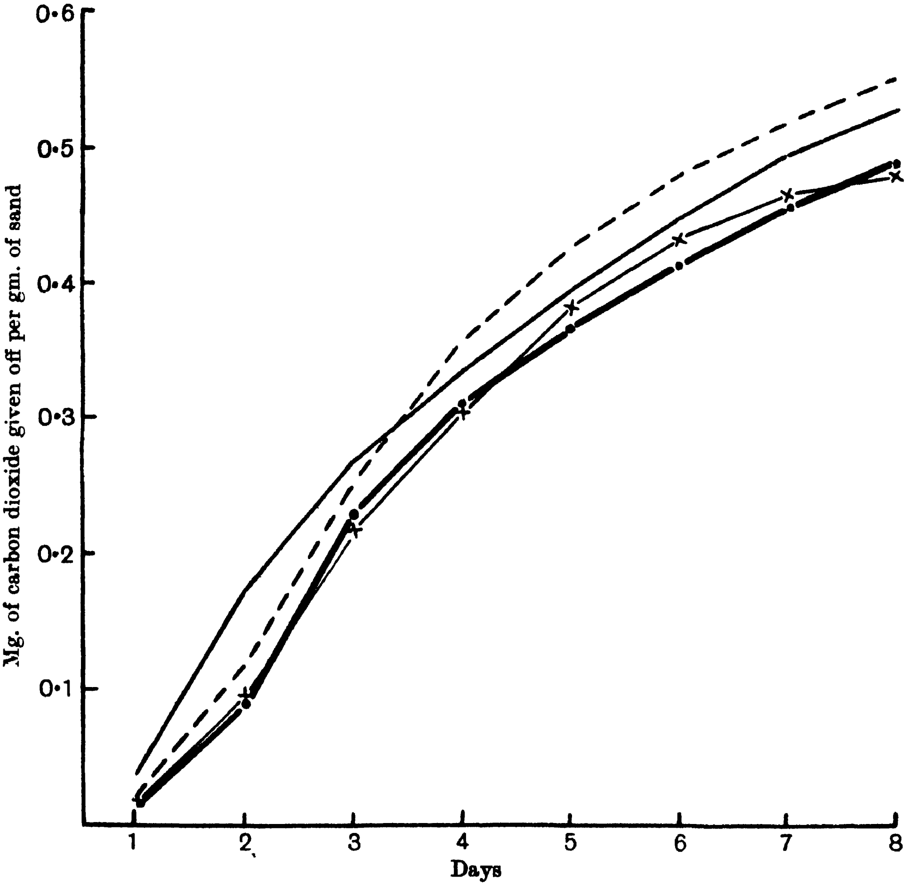

Fig. 3. Influence of repeated inoculation of protozoa. Four sand cultures used.

- - - - - Soil bacteria and protozoa mixed culture for all cultures.

——— As above + second inoculation at the beginning to second culture

●——● As above + second inoculation on the second day to third culture.

×——× As above + second inoculation on the fourth day to fourth culture.

Table VII.

The carbon dioxide production in 8 *days and the increase in the amount of carbon dioxide by the inoculation of protozoa.*

Maximum of producible CO$_2$	Produced amount of CO$_2$		Inoculation	Treatment
	in mg.	in %		
440	163·2	37·09	Mixed soil bacteria culture prepared from soil	Sand + peptone mineral solution
440	169·2	38·45		
440	220·0	50·00	Protozoa and bacteria mixed culture prepared from soil	
563	285·8	50·75		
440	152·2	34·59	"YB" bacteria	
440	190·9	43·39	"YB" bacteria and *Colpidium* sp.	
440	201·7	45·84		
440	216·6	49·23	Protozoa and bacteria mixed culture + "YB" bacteria and *Colpidium* sp.	
440	211·7	48·11	Protozoa and bacteria mixed culture and protozoa culture from a biological filter: (*a*) immediately	
440	194·2	44·14	(*b*) after 2 days	
440	192·2	43·68	(*c*) after 4 days	
440	146·0	33·18	Mixed soil bacteria	Sand + glucose $(NH_4)_2SO_4$ mineral solution
440	253·5	57·61	Protozoa and bacteria mixed culture	
440	253·4	57·59		
440	142·6	32·41	"YB" bacteria	
440	148·7	33·80		
440	220·5	50·12	"YB" bacteria + *Colpidium* sp.	

As it appears from Table VII the glucose ammonium sulphate media gave greater differences with the same C/N ratio than the peptone media, though according to Table III the bacterial population was more numerous in the latter case. The differences between the percentage carbon dioxide production of different cultures on the same medium are as follows:

		%
In peptone:		
Soil bacteria and protozoa mixed culture	Mixed soil bacteria	12·60
"YB" with *Colpidium* sp.	"YB" bacteria	10·02
In glucose and ammonium sulphate:		
Soil bacteria and protozoa mixed culture	Mixed soil bacteria	24·48
"YB" with *Colpidium* sp.	"YB" bacteria	17·02

It will be seen that on glucose-ammonium sulphate media, not only the absolute amount, but also the differences in the presence or absence of protozoa are nearly double those on the peptone media. Daily microscopic observation showed that the added protozoa established themselves in the media and therefore their respiration must have contributed to the carbon dioxide production, but cannot account for the whole of the increase. But the fact that by increasing still further the number of protozoa the production of carbon dioxide is now diminished, is evidence of some other factor operating to increase the carbon dioxide production. We have seen that this factor is *not* an increase in bacterial numbers. Unfortunately no data are available for the bacterial numbers after the second inoculation owing to an accident to the mechanism of the incubator, but it is almost certain that they would have been still further decreased. It is evident that a certain biological equilibrium exists between the bacteria and protozoa the optimum conditions of which are attained with soil bacteria + soil protozoa. If this equilibrium is affected in any direction, as occurred in these experiments with the extreme cases of the addition of more protozoa and of their absence, the percentage production of carbon dioxide is reduced.

PART II.

The previous investigations had shown that nearly double the amount of carbon dioxide was produced from glucose-ammonium sulphate media compared with that from peptone media of similar carbon nitrogen content; and also that there is a correspondingly greater difference in favour of the inoculation with bacteria + protozoa over the inoculation with bacteria alone.

No data, however, were obtained on the question of the effect of concentration of carbohydrate and of changes in the C/N ratio in the respiration of the micro-organisms. Cutler and Crump (3) made studies with 0·2 per cent. glucose with a C/N ratio of 10/1 and found an increase in respiration in the presence of protozoa, but previous workers appear to have neglected this question.

To the present writer it appeared that the importance of these two factors in soil processes was such as to call for further investigations on the influence of concentration of carbohydrate and of different C/N ratios on respiration in the presence or absence of protozoa.

METHODS.

The experiments were carried out in a similar manner to the previous ones, viz. sterile sand containing the nutrient media was inoculated with various cultures and the carbon dioxide produced was measured by Pettenkoffer's method. Bacterial counts were made daily and the presence of protozoa was controlled microscopically as described in detail in the previous section.

On account of the fixed C/N ratio of peptone it could only be used for investigations on the effect of changes in concentration. It was therefore necessary to confine these studies to the use of glucose and ammonium sulphate. Mineral salt solutions containing 0·2 and 0·6 per cent. glucose were prepared, and separately sterilised ammonium sulphate solutions were added so as to give C/N ratios of 20/1, 10/1, 5/1 and 3·5/1. For the inoculations the following cultures were used, viz. (*a*) bacteria + protozoa from Barnfield farmyard manured plot, (*b*) mixed soil bacteria prepared as previously mentioned, (*c*) pure cultures of "YB" bacteria.

RESULTS.

In the first experiment solutions of 0·2 per cent. glucose with ammonium sulphate equivalent to C/N ratio of 20/1, 10/1, 5/1 and 3·5/1 were inoculated with mixed cultures of protozoa and bacteria. The production of carbon dioxide calculated in mg. per gm. of sand and numbers of bacteria are plotted as curves in Fig. 4.

The curves for carbon dioxide production reached a maximum on the second day, followed by the curves of bacterial numbers a day later, in all cases except the C/N ratio 20/1, in which the two curves revealed a maximum simultaneously. With an increase of the C/N ratio the production of carbon dioxide increased.

The second experiment used the same media but omitting the C/N ratio 5/1. The inoculation consisted of mixed soil bacteria without protozoa. The results are plotted in Fig. 5.

Comparing these curves with the previous curves in Fig. 4 not only is the total amount of carbon dioxide less but the difference between the curves of the various C/N ratios is not significant.

With regard to the curves of bacterial numbers the normal curve is shown only by the one with C/N ratio of 3·5. In the case of the curves of decreasing C/N ratio there are two maxima.

In the third experiment 0·6 per cent. glucose was used with C/N ratios of 20/1, 10/1 and 3·5/1 inoculated with soil bacteria without protozoa.

The results are shown in Fig. 6. These curves of carbon dioxide production are remarkable in showing no significant differences between total yields from the various C/N ratios. The curves for bacterial numbers show two maxima in the case of C/N ratio 20/1, with the C/N ratio 10/1 the bacterial numbers and carbon dioxide production reach their maxima simultaneously and in the case of the C/N ratio 3·5/1 the bacterial numbers reach a maximum a day later.

This experiment was repeated under similar conditions using an inoculation of "YB" bacterium in pure culture.

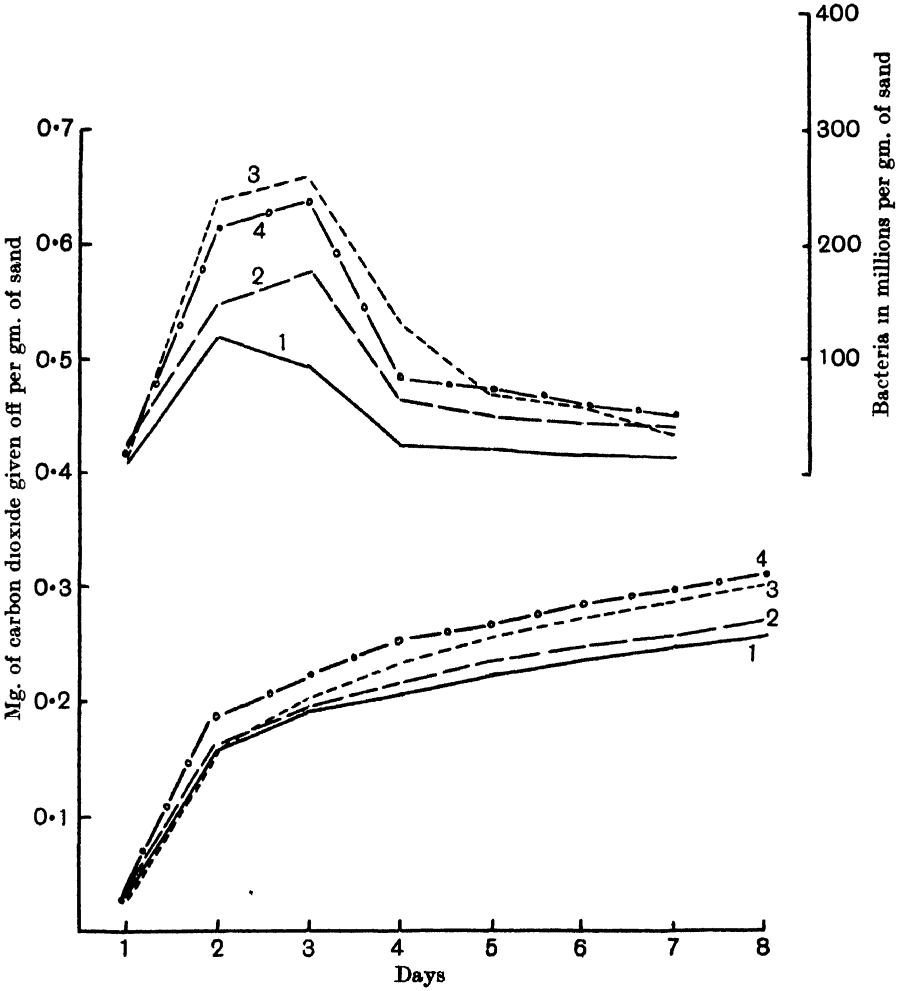

Fig. 4. Influence of C/N ratio on the CO_2 production. 0·2 per cent. glucose, soil bacteria and protozoa mixed culture.

——— (1) C/N = 20/1. — — — — (2) C/N = 10/1.
- - - - - (3) C/N = 5/1. o—o—o (4) C/N = 3·5/1.

The results shown in Fig. 7 are generally similar to the previous results except that the increasing of the C/N ratio reduced slightly the carbon dioxide production. The culture itself also appeared to have been less efficient than the mixed population used in the previous experiments.

The bacterial curves for the greater C/N ratio give the normal curve, but the lesser ratios give greater fluctuations.

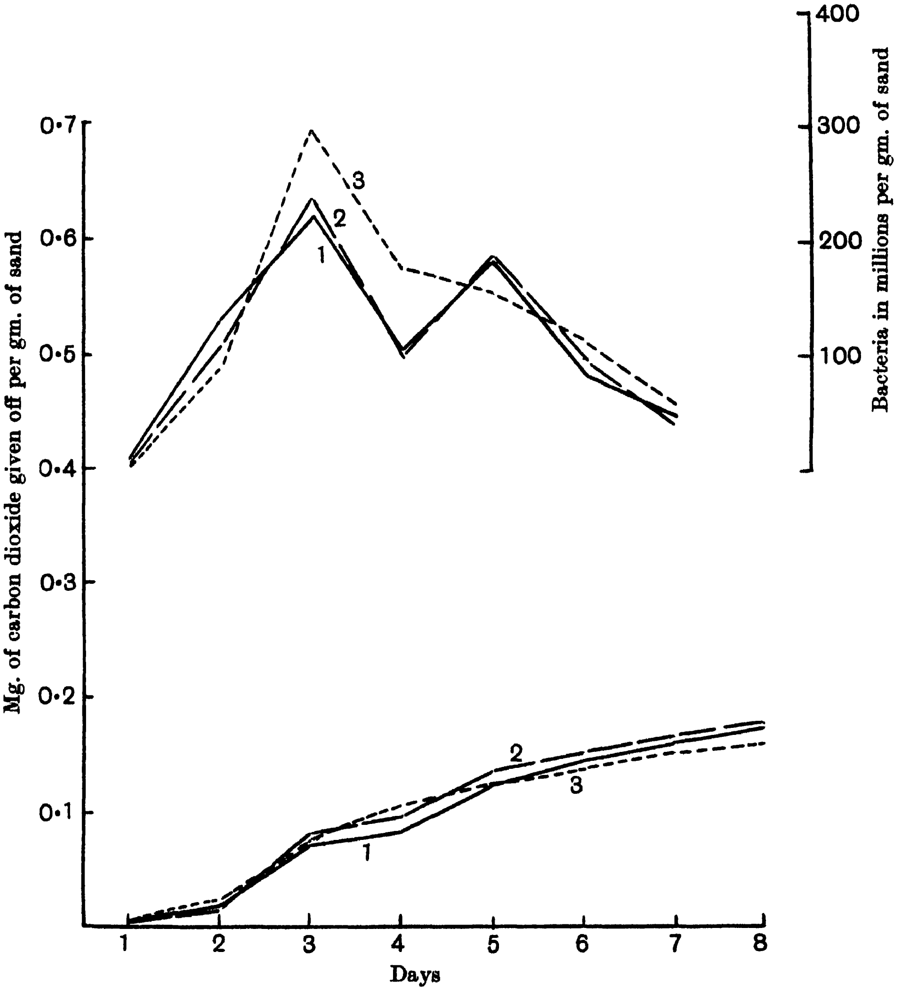

Fig. 5. Influence of C/N ratio on the CO_2 production. 0·2 per cent. glucose, soil bacteria alone.

——— (1) C/N = 20/1. — — — (2) C/N = 10/1. - - - - - (3) C/N = 3·5/1.

The fifth experiment was carried out with mixed bacteria and protozoa and the same media as in experiment three, viz. 0·6 per cent. glucose and C/N ratios 20/1, 10/1 and 3·5/1. Increasing the C/N ratio resulted in increased carbon dioxide production, and the total amount of carbon dioxide produced compared with that obtained in the previous experiments with bacterial cultures alone is much greater (Fig. 8).

The bacterial curves show less fluctuations and reach a maximum later in the narrower C/N ratios of 10/1 and 3·5/1.

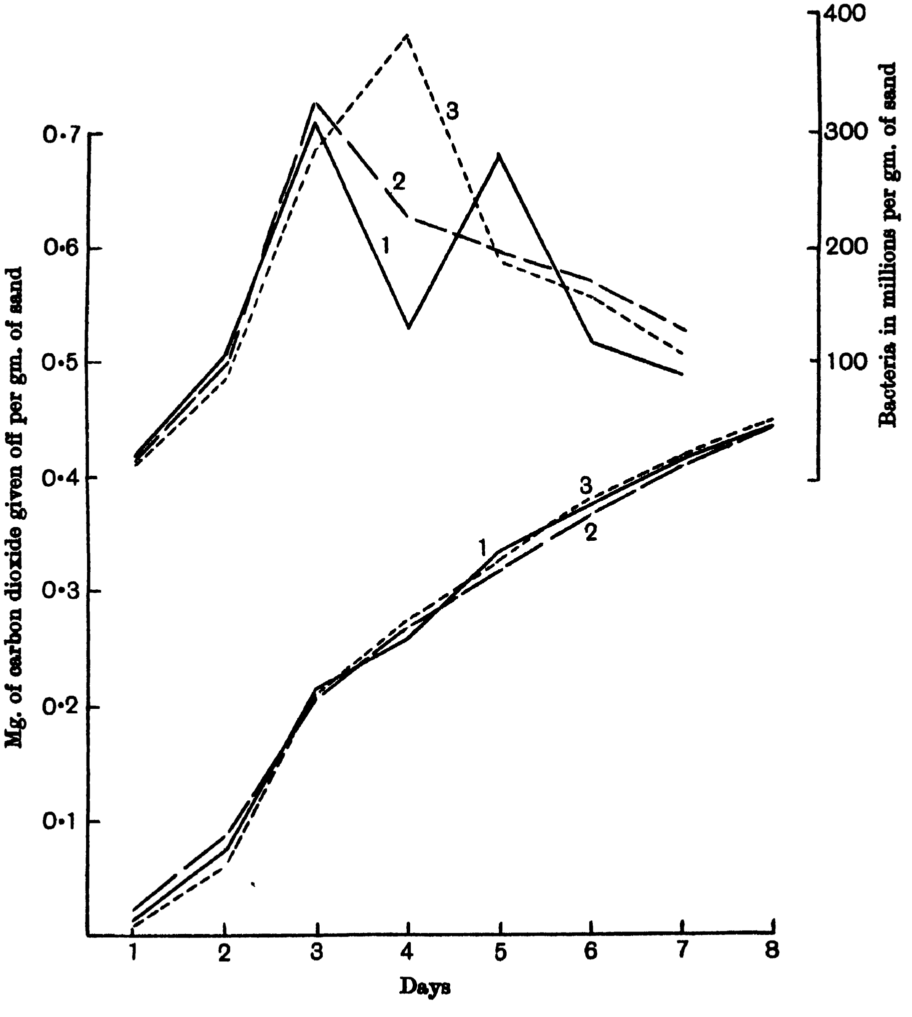

Fig. 6. Influence of C/N ratio on the CO_2 production. 0·6 per cent. glucose, soil bacteria alone.

——— (1) C/N = 20/1. — — — (2) C/N = 10/1. - - - - - (3) C/N = 3·5/1.

The results of all these experiments are collected in Table VIII, in which the maximum amount of carbon dioxide producible from the sugar is expressed as 100 and the amounts of carbon dioxide actually obtained are given as percentages.

Table VIII.

The carbon dioxide production in 8 *days and the increase in the amount of carbon dioxide by the inoculation of protozoa.*

Maximum of producible CO_2	Produced amount of CO_2		Inoculation	Treatment	
	in mg.	in %			
146·5	68·9	47·03	Mixed soil bacteria culture prepared from soil	Sand—mineral solution 0·2 % glucose and different amounts of $(NH_4)_2SO_4$	C : N 20 : 1
	69·8	47·65			C : N 10 : 1
	63·4	43·28			C : N 3·5 : 1
	101·5	69·28	Protozoa and bacteria mixed culture prepared from soil		C : N 20 : 1
	106·9	72·82			C : N 10 : 1
	120·3	82·11			C : N 5 : 1
	124·3	84·85			C : N 3·5 : 1
440	177·9	40·43	Mixed soil bacteria	Sand—mineral solution, 0·6 % glucose and different amounts of $(NH_4)_2SO_4$	C : N 20 : 1
	177·6	40·41			C : N 10 : 1
	170·5	40·80			C : N 3·5 : 1
	172·1	39·11	"YB" bacteria		C : N 20 : 1
	167·9	38·16			C : N 10 : 1
	165·4	37·59			C : N 3·5 : 1
	277·0	62·95	Protozoa and bacteria mixed culture		C : N 20 : 1
	289·6	65·82			C : N 10 : 1
	296·5	67·38			C : N 3·5 : 1

Comparing the two sections of the table for total carbon dioxide production it will be seen that:

(1) In every case the total percentage of carbon dioxide from similar cultures is greater with 0·2 per cent. glucose solution than with 0·6 per cent.

(2) With 0·2 per cent. glucose varying the C/N ratio from 20/1 to 3·5/1 results in an increase in total carbon dioxide production in favour of the presence of protozoa of from 22·25 to 45·57 per cent.

(3) But, with 0·6 per cent. glucose similar variation in the C/N ratio showed an increase in total carbon dioxide production in the presence of protozoa of 22·52–26·58 per cent., showing that at a higher concentration of carbohydrate the stimulating effect of the protozoa on respiration is less marked.

Finally, comparing the figures for carbon dioxide production of bacterial cultures alone, it was found that varying the C/N ratio from 20/1 to 3·5/1 did not result in any significant difference in the carbon dioxide production, the tendency, if any, was in the direction for slight reduction especially in the case of the pure cultures of "YB" bacteria.

In the first part of the paper it was shown that comparing cultures in 0·5 per cent. peptone and 0·6 per cent. glucose-ammonium sulphate of equivalent C/N ratio (3·5) and carbon content, the presence of protozoa caused a greater increase in the amount of carbon dioxide produced from the glucose than from the peptone media.

The second part of these investigations showed that this effect is still more pronounced with lower concentrations of glucose at all investigated C/N ratios.

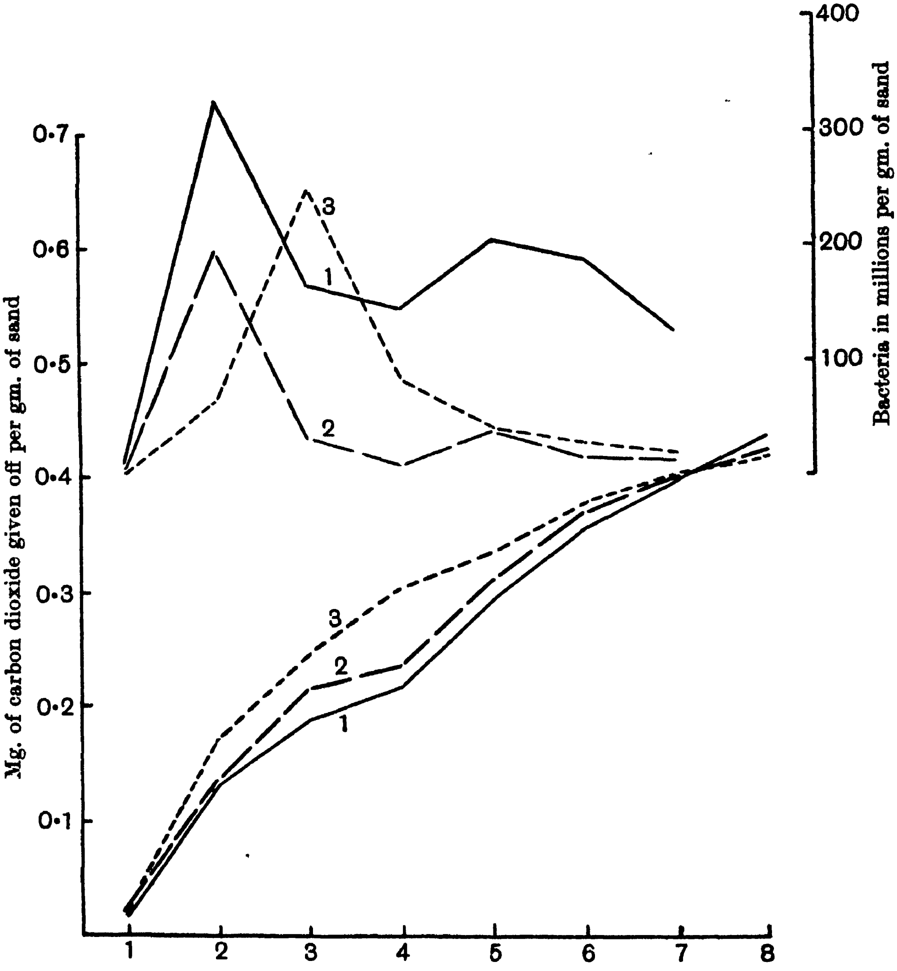

Fig. 7. Influence of C/N ratio on the CO_2 production. 0·6 per cent. glucose, "YB" bacteria pure culture.
——— (1) C/N=20/1. — — — (2) C/N=10/1. - - - - - (3) C/N=3·5/1.

It has been shown that in bacterial cultures without protozoa the increase of the C/N ratio had no effect or only a slight depressing effect on the carbon dioxide production, whereas in the presence of protozoa there is a very pronounced increase.

From a consideration of the bacterial curves it is seen that the correlation which normally exists between carbon dioxide production and bacterial numbers in pure cultures is of a less degree in the presence

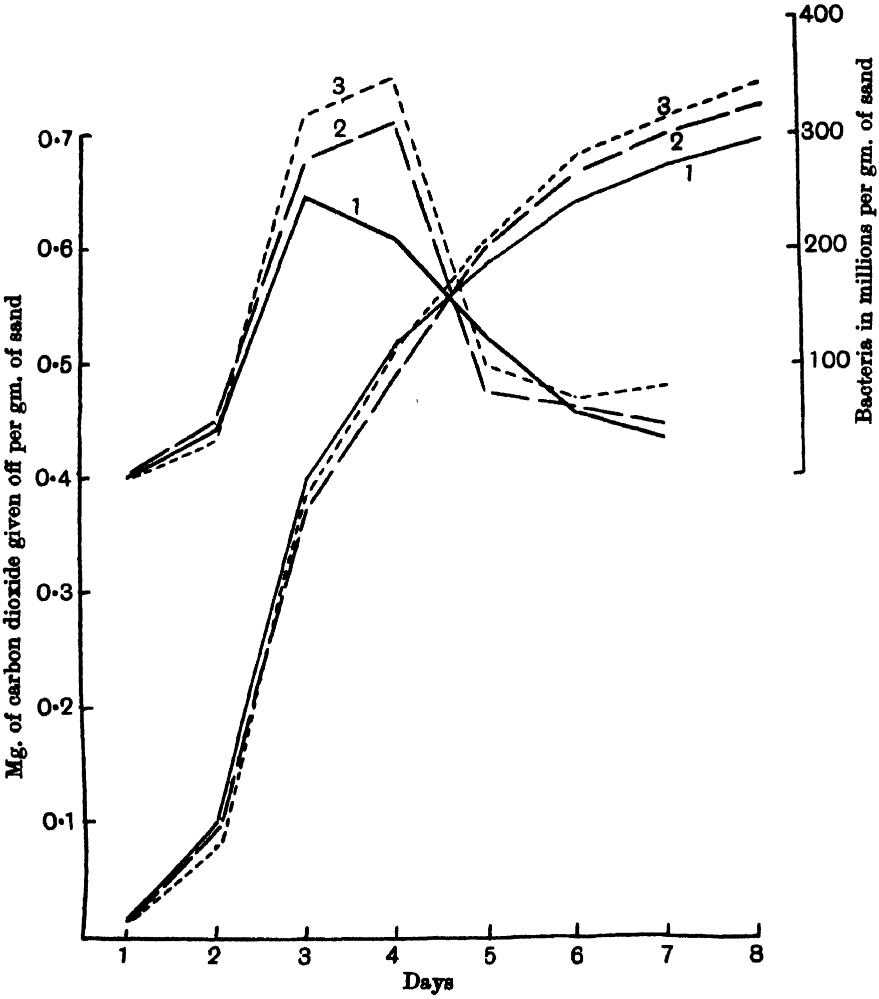

Fig. 8. Influence of C/N ratio on the CO_2 production. 0·6 per cent. glucose, soil bacteria and protozoa mixed culture.
——— (1) C/N = 20/1. — — — (2) C/N = 10/1. - - - - - (3) C/N = 3·5/1.

of protozoa in glucose-ammonium sulphate media. In the latter case the density of the bacterial population is almost the same as without protozoa, whilst the carbon dioxide production is actually much greater.

In bacterial cultures either of pure "YB" or mixed soil bacteria, the correlation between numbers and carbon dioxide production is disturbed with a C/N ratio less than 10/1. As already pointed out with a low C/N ratio the rise in bacterial numbers shows two maxima.

These fluctuations are accounted for by the shortage of nitrogen which temporarily arrests the bacterial growth until a re-assimilation of the combined nitrogen from dead protoplasm can take place resulting in a second rise in bacterial numbers with a corresponding carbon dioxide production.

In this connection it is interesting to note that on percolating filters with solutions of 0·2 per cent. sucrose-ammonium sulphate Barritt[1] observed that the nitrogen requirement of a mixed population was supplied by a C/N ratio of 15, and that with a wider C/N ratio the percentage purification was reduced.

SUMMARY.

Experiments have been carried out on carbon dioxide production from sand cultures with peptone and glucose solution inoculated with various types of bacteria and protozoa, and with glucose and ammonium sulphate solutions of varying concentrations and C/N ratio. The following results were obtained:

1. The presence of protozoa increases the carbon dioxide production especially in mixed bacterial cultures.
2. The increase of carbon dioxide production is greater in glucose solution than in peptone.
3. A further increase in the number of protozoa has an unfavourable effect on the carbon dioxide production.
4. The number of bacteria is smaller in the presence of protozoa than in their absence, but the bacterial efficiency is greater and more uniform.
5. The bacterial numbers and carbon dioxide production are definitely correlated in peptone, but in glucose to a less degree especially in the presence of protozoa.
6. The reduction of concentrations of glucose from 0·6 to 0·2 per cent. resulted in a greater percentage production of carbon dioxide.
7. With a lower concentration (0·2 per cent.) of glucose the presence of protozoa causes a greater increase in carbon dioxide production than in higher concentrations (0·6 per cent.).
8. In the absence of protozoa increasing the C/N ratio had no or only a slight depressing effect on carbon dioxide production.
9. In the presence of protozoa increasing the C/N ratio is followed by a marked increase in carbon dioxide production.

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[1] *Biochem J.* 1931, xxv, 4, 187, 1419.

10. In bacterial cultures a lessening of the C/N ratio below 10/1 results in a fluctuation of bacterial numbers.

I am indebted to Sir John Russell for his kindness in giving me facilities to carry out the work in this Institution. The work was done in Mr D. Ward Cutler's department, and I take this opportunity of expressing my gratitude for his ever ready help and unfailing kindness. For the culture of *Colpidium* I am greatly indebted to Miss Jane Meiklejohn of the same department.

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INFECTION BY BACT. RADICICOLA IN RELATION TO THE MICROCHEMISTRY OF THE HOST'S CELL WALLS.

BY

E. McCOY, Ph.D.

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Infection by Bact. Radicicola *in Relation to the Microchemistry of the Host's Cell Walls.*

By ELIZABETH McCOY, Ph.D.

(Communicated by Sir John Russell, F.R.S.—Received February 19, 1932.)

(Department of Bacteriology, Rothamsted Experimental Station, Harpenden.)

[PLATE 26.]

The intimate relationship of the nodule bacteria to their hosts, the Leguminosæ, offers some fascinating problems in plant chemistry, which have been only partially solved by the researches of the past 50 years. These investigations have been mainly botanical and bacteriological, and such chemical work as there is has been done from an agricultural standpoint, with a view to discovering and explaining the fertilising action of leguminous crops. Consequently our hypotheses of the more detailed physiological chemistry of the nodule have been deduced largely from cytological and histological evidence. The microchemical mode of attack has been almost entirely neglected.

Even though methods in botanical microchemistry are still very unsatisfactory, it seemed possible that a microchemical study of nodule tissues might throw new light on the nodule problem. The results to be reported here are admittedly qualitative and, in some cases, not conclusive, but the author feels that her expectation was justified, and that the field is a fertile one for the future. In general, the methods used were based on those of Molisch (1923) and Tunmann (1913) and on an outline of methods by Dr. Sophia Eckerson (unpublished notes lent by an associate). Additional methods, based on known properties of the substances in question, were devised when necessary.

The work has been done on tissues of lucerne, pea, clover, and broad bean seedlings and nodules. Lucerne tissue has been the most extensively used, and unless otherwise indicated, the results described will be those relating to this plant. The investigation has been directed along two related lines, which will be considered separately.

PART I.—THE INFECTION OF ROOT HAIRS.

The Chemical Composition of the Cell Walls of Normal Root Hairs.

Despite the importance of the root hairs in the life of the plant, it is surprising that there has been very little work on the chemical composition of their cell walls. Such results as have been published are decidedly contradictory; whether because of actual differences among the plants studied, or because of unreliable methods of testing, it is difficult to say. It is not even certain that cellulose is a common constituent of the root-hair wall; Roberts (1916) found it present as an inner layer, but Howe (1921) found none at all and in its place a layer of callose. Addoms (1927) obtained uncertain results with cellulose tests.

In the hairs of the leguminous plants here studied, cellulose is undoubtedly present, even to the extreme tips of the hairs. It is true that a direct test on fresh tissue, with either chlor-zinc-iodide* or the hydrocellulose test† reagents, gives a nondescript yellowish green colour, which is inclined to fade within a few hours, although cortical parenchyma, phloem, and even young vascular elements are characteristically blue, as for cellulose. But the cellulose tests are strongly positive on root hairs, which have been treated (1) in boiling 5 per cent. NaOH or KOH from a half to 6 hours, or (2) in boiling 3 per cent. HCl 1 hour followed by boiling 5 per cent. KOH 1 hour, or (3) in cold eau de Javelle‡ 2 to 10 days. It will be shown later that these treatments remove an extremely resistant hemicellulose, which is responsible for the failure of the usual cellulose stains to act directly upon root hairs. The final and convincing proof of the presence of cellulose in the root hairs will be seen in the following survival and solubility tests :—

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* Schultze's reagent from the British Drug Houses was used throughout these tests for the sake of uniformity of reagent.

† The hydrocellulose tests were done with I_2KI solution (I_2 0·3 gm., KI 0·75 gm., H_2O 50 c.c.) and H_2SO_4 66 or 75 per cent. (Eckerson).

‡ Strasburger formula—20 c.c. of 25 per cent. calcium hypochlorite made to 100 c.c. with water; 100 c.c. of 15 per cent. aqueous K_2CO_3.

1. Tissue treated in boiling 3 per cent. HCl 1 hour, followed by boiling 5 per cent. KOH 1 hour and examined in water mount, shows root hairs intact, though extremely hyaline. They are instantly dissolved in copper-oxide-ammonia reagent* diffused under the coverglass.
2. Tissue boiled in 5 per cent. KOH alone also shows surviving membranes, soluble in copper-oxide-ammonia.
3. Tissue extracted with boiling 0·5 per cent. $(NH_4)_2C_2O_4$ 1 to 6 hours, preceded or followed by cold eau de Javelle 2 to 10 days, shows the remainder of the root-hair walls easily soluble in copper-oxide-ammonia.
4. Tissue treated with boiling 0·5 per cent. $(NH_4)_2C_2O_4$ 1 to 6 hours, followed by boiling 5 per cent. KOH 1 hour, contains root hairs easily soluble in copper-oxide-ammonia.
5. Tissue treated in boiling 5 per cent. KOH 1 hour, followed by boiling 3 per cent. $H_2C_2O_4$ 1 hour, and either boiling 5 per cent. KOH or boiling 5 per cent. Na_2CO_3 for 1 hour, shows persisting root hairs easily soluble in copper-oxide-ammonia.

All these methods should remove both pectic and hemicellulosic materials and leave any residual cellulose until the copper-oxide-ammonia treatment These tests have been repeated many times on lucerne, clover, pea, and broad bean tissues, and cellulose has been found consistently even to the tips of both young and old hairs.

Of the pectic materials in the root hairs, little can be said. Calcium pectate has been shown by its formation of CaC_2O_4 crystals upon treatment with either $(NH_4)_2C_2O_4$ or $H_2C_2O_4$. Roberts and Howe have reported that the calcium pectate forms the outer layer of the wall, so that the crystals in oxalic-acid-treated material would lie in an outer layer of insoluble pectic acid. If this were so in the leguminous plants here studied, the crystals should be liberated by the removal of the pectic acid as, for instance, by formation of its sodium or potassium salt. But one can remove all pectic acid by treatment with boiling 5 per cent. Na_2CO_3 without disturbing the imbedded crystals, which are, however, set free upon dissolution of the cellulosic residue with copper-oxide-ammonia. This behaviour indicates that the original calcium pectate occurred not in a separate layer but in association with the cellulose-containing layer or layers of the root-hair wall. There is evidently some variation in the amount of calcium pectate present, as judged from the relative abundance of crystals. No regularity in this respect could be established, either in relation to the kind of plant or the age and condition of tissue. Howe reported pectose "especially found in young tissue." Whether pectose, pectin, or pectic acid is present in the leguminous root hairs cannot be decided from the data available. The difficulty arises owing to the simultaneous presence of hemicellulose, which is also stainable by ruthenium red. Furthermore, all reagents which remove the hemicellulose also remove the pectic materials, and thus it is not possible to differentiate by subsequent staining.

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* Made according to the method in the Eckerson manual from copper filings treated with excess ammonia and allowed to stand open to the air until the reagent is of sufficient strength to dissolve cotton fibres instantaneously. The solution deteriorates in 4–6 weeks and must be replaced.

The converse extraction, pectic material from hemicellulose-cellulose residue, is, of course, possible with boiling ammonium oxalate, or oxalic acid followed by such weak alkali as 5 per cent. Na_2CO_3, which does not affect the hemicellulose. The first hint of the presence of constituents other than cellulose and the pectic substances, in fact came from tests upon root hairs of oxalate-treated material. Such root hairs were only poorly stained by Schultze's reagent, but deeply stained by ruthenium red, and not entirely dissolved by copper-oxide-ammonia. After treatment with boiling dilute caustic alkali (5 per cent. KOH), eau de Javelle in the cold or concentrated NH_4OH in the cold, the offending substance was removed. There are several substances which might account for some or all of those properties. Suberin, if present, might hinder the cellulose reaction; would be insoluble in copper-oxide-ammonia; but soluble in dilute alkali. But the root hairs are not stained by Sudan III or Scharlach R; are soluble in cold 50 per cent. chromic acid, and give neither the ceric acid nor phellonic acid tests. The failure of Sudan III to stain the hairs eliminates the possibility of there being a fatty substance, as does also the fact that ether extraction failed to remove the resistant substance. Callose would account for the insolubility in copper-oxide-ammonia and perhaps for the failure of the cellulose test; also, Howe reported callose in root hairs of several plants, including the pea and garden bean. Her report is based upon positive staining of the root-hair membrane with resorcin blue. In the present work no trace of callose could be shown by either resorcin blue or aniline blue staining. Nor did the specific solubility tests for callose by cold 1 per cent. KOH, $CaCl_2$, $SnCl_2$, or glycerine at 28° C. give any indication of its presence.

Another class of compounds which might conceivably account for the observed reactions of the root hairs are the proteins. There was indication of their presence in the walls of hairs treated with Millon's, xanthoproteic, or biuret test reagents, but it seemed possible that small quantities might be present and yet escape detection by staining methods. Solubility methods were therefore applied. Proteins in general are either acid-soluble or alkali-soluble, whereas the resistant substance of the root hairs survives both prolonged boiling in 3 per cent. HCl (1 to 6 hours) and extraction with weak alkalies such as 0·5 to 1 per cent. caustic alkali with boiling for 1 to 2 hours, 5 per cent. alkaline carbonate boiling 1 to 2 hours, or the ammonia of copper-oxide-ammonia in the cold for 10 days. Furthermore, digestion with proteolytic enzymes, pepsin in 0·2–0·3 per cent. HCl, or trypsin at p_H 8·0, fails to remove the inhibitory substance. And lastly, the root hairs do not give the Congo red-acid reaction which Priestley and Tupper-Carey (1922) attributed to protein in the walls of root and stem meristems. This reaction will be further discussed in relation to the nodule meristem in Part II of this paper.

The resistant substance cannot be lignin, since the root hairs, fresh or otherwise, fail to give any of the colour tests for lignin. Neither do they contain pentoses or pentosans in quantity to give the orcinol or phloroglucinol tests. They are also negative to the iodine test for amyloid, applied directly or after treatment of the tissue with eau de Javelle. This is contradictory to the finding of Ziegenspek (1920) who detected amyloid in the tips of the root hairs, which he studied, and considered it to be a transitional product in the formation of cellulose.

The only substances which have been found in the root hairs of the leguminosæ, then, are cellulose, calcium pectate (probably other pectic substances also) and a comparatively resistant material whose properties are as follows: solubility in caustic alkali (3–5 per cent.) with boiling 1 hour; in concentrated ammonia and in eau de Javelle slowly in the cold; insolubility in 0·5–1 per cent. caustic alkali with boiling ½ to 2 hours; 5 per cent. alkaline carbonate; 3 per cent. hydrochloric acid; 3 per cent. sulphuric acid; 0·5 per cent. ammonium oxalate; 3 per cent. oxalic acid alone or followed by 5 per cent. sodium carbonate, or copper-oxide-ammonia. It is stained by aqueous ruthenium red, iodine green, and some 12 other basic dyes tested; it is stained poorly or not at all by acid dyes. It is coloured yellow by Schultze's reagent, and its presence with cellulose prevents the latter from showing its characteristic blue colour with this reagent. It is not destroyed by heating to 210° or 300° C. in pure glycerine, as recommended by Schorger (1926) and Molisch (1923) for distinguishing between hemicellulose and true cellulose. With the last-mentioned exception, the substance has the usual properties of a hemicellulose. It has been found in the tips as well as in the lateral walls of the root hairs of the pea, lucerne, broad bean and clover.

The Phenomena of Infection.

It is a curious fact that infection of a root hair by the nodule organism always occurs at the tip, occasionally at the tip of a lateral branch. And yet, so far, it has not been possible to find any chemical difference in the walls at those points, unless it be a slight concentration of the hemicellulose constituent. There is, however, some evidence of a physical difference. When seedlings of lucerne and clover, grown in petri dishes on the surface of mineral-salts agar, were watered with sterile distilled water, their exposed root hairs swelled out into queer club-shaped and bulbous forms, the tips being first affected. When sterile plant nutrient solution was used, deformation of the hairs did not occur. This is in accord with the observations of Stiehr (1903), who reported that the tips of root hairs are first to respond to changes in osmotic relations, and that when hairs are immersed in distilled water, the bursting invariably occurs at their tips. It is therefore suggested that the tips, most readily permeable to water, are also most readily penetrated by the bacteria.

Many investigators have seen and described the infected root hair, but none has described the exact mechanism of the entrance of the bacteria. There would seem to be two possibilities: that the bacteria enter mechanically injured or broken root hairs, or that they enter by secreting some substance capable of dissolving the wall at the point of entry. Proof of either alternative hinges upon whether the well known curling of an infected hair is induced by the bacteria, or whether the organisms infect a hair deformed from some other cause. It is known that the form of root hairs varies somewhat with the medium in which the plant is grown. Roots in moist air generally produce an abundance of straight cylindrical hairs, while those in soil or sand bear hairs more or less distorted by contact with the solid particles. Such contacts might be supposed to produce injuries through which bacteria could enter. To determine whether the bacteria themselves induce a further distortion of the hairs, the numbers of bent and curled hairs on plants in the presence and in the absence of the bacteria were compared. The data shown in Tables I and II are selected from a large number of such counts made upon plants grown in pots of sterilised sand. The presence of the bacteria is associated with a very significant increase in the proportion of bent and curled hairs In agar-tube cultures, where the factor of mechanical injury is negligible, the results are even more striking, Tables III and IV. The bacteria, therefore, do not rely upon accidentally deformed root hairs but are in some way able to act upon the root hairs. Further evidence of this was obtained by the following experiments.

Table I.*—Pot culture controls. Lucerne, age 3 weeks.

Pot No	Plant	Differential counts of root hairs			
		Straight	Bent	Curled.	Infected
		per cent	per cent	per cent	per cent
1	I	73	27	0	0
	II	78	22	0	0
2	I	74	25	1	0
	II	67	32	1	0
3	I	74	26	0	0
	II	77	22	1	0
4	I	75	25	0	0
	II	83	17	0	0
Mean per cent		75 125	24 5	0 375	0
Standard error		±1 62	±1 55	—	—

Table II—Pot cultures of lucerne inoculated with lucerne bacteria Age 3 weeks.

Pot No	Plant.	Differential counts of root hairs			
		Straight	Bent	Curled	Infected †
		per cent	per cent	per cent	per cent
1	I	41	45	14	2
	II	44	43	13	2
2	I	37	44	19	1
	II	38	48	14	4
3	I	43	46	11	3
	II	39	45	16	3
Mean per cent		40 33	45 166	14 5	2 5
Standard error		±1 16	±0 71	±1 12	±0 18

† The infected hairs, all being curled, are included in the previous column

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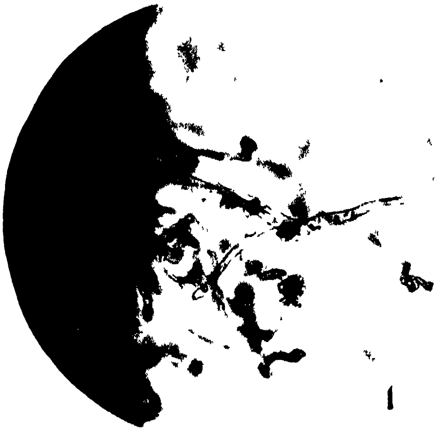
* The percentages in all of the following tables are based upon counts of 20 consecutive hairs at each of five random portions of root from each plant studied.



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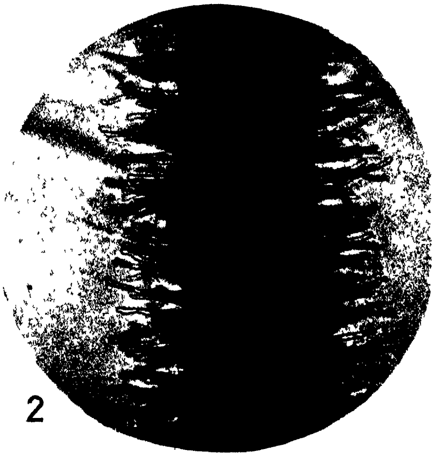

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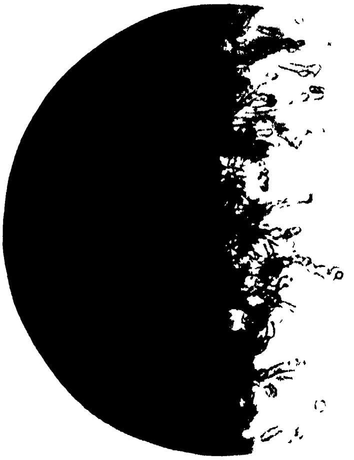

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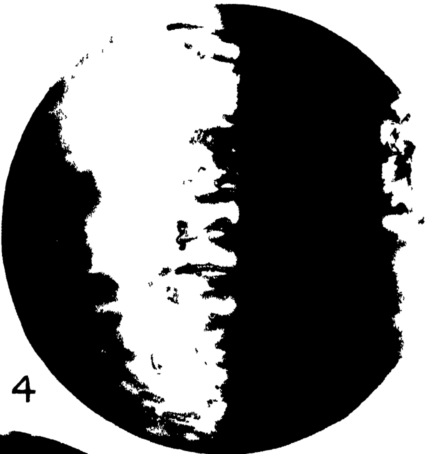

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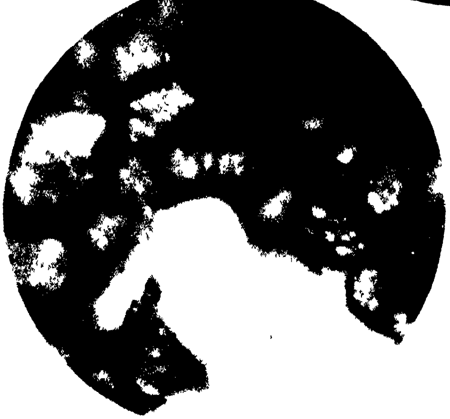

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Table III.—Tube-culture controls. Lucerne, age 3 weeks.

Tube No.	Plant.	Differential counts of root hairs.			
		Straight.	Bent.	Curled.	Infected.
		per cent.	per cent.	per cent.	per cent.
1	I	92	8	0	0
	II	93	7	0	0
2	I	95	5	0	0
	II	95	5	0	0
Mean per cent.		93·75	6·25	0	0
Standard error		±0·75	±0·75	—	—

Table IV.—Tube cultures of lucerne inoculated with lucerne bacteria. Age 3 weeks.

Tube No.	Plant.	Differential counts of root hairs.			
		Straight.	Bent.	Curled.	Infected.
		per cent.	per cent.	per cent.	per cent.
1	I	53	38	9	1
	II	52	37	11	0
2	I	57	38	5	0
	II	55	40	5	0
Mean per cent.		54·25	38·25	7·5	0·25
Standard error		±1·11	±0·63	±1·5	—

Some 16 cross-inoculation groups among nodule bacteria are known. Bacteria of any one group are not able to form nodules on the plants of any other group. Do the bacteria fail to produce the preliminary curling of the hairs, do they fail to enter the hairs or, once inside, do they find the environment unfavourable ? It seemed possible that study of non-infection might lead to a better understanding of infection. Some cross-inoculation experiments were therefore set up in both sand-pot and agar-tube culture, and a study was made of the root-hairs produced. The following cross-inoculations were studied :—

Clover plants × lucerne bacteria.

Lucerne plants × clover bacteria.

Pea plants × clover bacteria.

Pea plants × lucerne bacteria,

The root hairs were carefully examined, but no granules which might be presumed to be bacteria were seen within the hairs. Furthermore, neither infection threads nor abortive nodules were produced by the foreign organisms, but the curling reaction of the root hairs was unusually marked. The hairs were often so crimped, branched, curled, and matted together that it was impossible to count separately those curled at the tips, as if infected, and those otherwise deformed, called bent. Tables V and VI and fig. 1 show results of the pea plant × lucerne bacteria cross. The other crosses gave the same reaction in more or less degree, indicating that the reaction is a general one.

Table V.—Pot-culture controls. Peas, age 1 month.

Pot No.	Plant.	Differential counts of root hairs.	
		Straight.	Bent and curled.
		per cent.	per cent.
1	I	93	7
	II	86	14
2	I	94	6
	II	90	10
3	I	90	10
	II	88	12
4	I	96	4
	II	91	9
Mean per cent		91	9
Standard error		±1·15	±1·15

Table VI.—Pot cultures of peas inoculated with lucerne bacteria. Age 1 month.

Pot No.	Plant.	Differential counts of root hairs.	
		Straight.	Bent and curled.*
		per cent.	per cent.
1	I	27	73
	II	20	80
2	I	26	74
	II	21	79
3	I	28	72
	II	17	83
Mean per cent.		23·16	76·83
Standard error		±1·81	±1·81

* Infected hairs, none.

The fact that infection threads were not found in the abnormally curled root hairs does not, of course, prove that the bacteria did not penetrate the cell walls; they might have been killed upon reaching the protoplast. Neither does it tell whether the curling reaction is a response to external attack by the bacteria or to some, perhaps toxic, stimulus set up after their entrance. To test these possibilities it was decided to do an experiment similar to one reported by Hiltner in 1900. He found that a filtrate of a culture passed through a Chamberland filter exerts a softening effect upon root hairs and causes a typical curvature of them. The test was made upon clover and lucerne plants in agar-tube culture. Six tubes of each set were treated at the time of planting with 5 c.c. per tube of filtrates* from 7 day-old cultures as follows :—

Clover plants × filtrate of clover bacteria.
Clover plants × filtrate of lucerne bacteria.
Lucerne plants × filtrate of lucerne bacteria.
Lucerne plants × filtrate of clover bacteria.

Control tubes were prepared with sterile tap water in simulation of the moisture conditions of the test series. Figs. 2, 3 and 4, Plate 26, are photomicrographs of the results, taken when the plants were 3 weeks old. Curling as well as other deformation of the root hairs, including branching (note especially fig 4), were produced in the absence of the bacterial cells. The results indicate that the curling which normally precedes infection of the root hairs is produced by a substance secreted by the bacteria before they enter.

These root hairs curled by bacteria-free filtrates, those curled by bacteria of other cross-inoculation groups, and hairs actually containing infection threads were repeatedly tested for the constituents of the normal root-hair wall, namely cellulose, pectic materials (especially calcium pectate) and the resistant hemicellulose. All were found, even in the curled tips. There was absolutely no indication that the bacterial action had produced any chemical change in the walls. This raises the question as to how the bacteria penetrate the walls of the root hair.

Since cellulose is present in the root-hair wall, cultures of the bacteria were tested upon various cellulose media: lucerne root-extract agar containing ground filter paper; similar agar with strips of sterile filter paper laid upon the surface of the agar and inoculated by streaking across the strip so as to insure direct contact of the bacteria with the cellulose; similar agar containing native cellulose prepared from lucerne root-meal, and all of the above media with 0·25 per cent. of sucrose to ensure initial growth of the bacteria. In no case was there any visible attack on the surrounding cellulose nor any indication of attack even close to or under the line of growth, when the plates were tested for cellulose by the hydrocellulose and chlor-zinc-iodide tests.* These findings agree with Beijerinck's original report (1888) on the absence of any cellulose-dissolving enzyme in his *B. radicicola.*

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* The filtrates were prepared from suspensions of the bacteria in sterile tap water by Berkefeld filtration, and were proved bacteria-free by test of 1 c.c. portions on slopes of root-extract agar and by the fact that neither infection threads nor nodules were formed on the test plants of homologous group.

The action of the nodule bacteria on other constituents of the root hairs, such as the pectic materials, remained to be investigated, since no previous report on the subject could be found. Root extract media were prepared (1) with no added carbohydrate, (2) with 0·5 per cent. of apple pectin,† (3) with apple pectin plus 0·5 per cent. of sucrose, (4) with 0·5 per cent. of lemon pectin,‡ (5) with lemon pectin plus sucrose, (6) with 0·5 per cent. calcium pectate,§ and (7) with calcium pectate plus sucrose. The pectins were sterilised separately in distilled water solution and were added aseptically to the media as required. The p_H of the finished media ranged from 6·8 to 7·2. In all, 17 cultures of the nodule bacteria representing various cross-inoculation groups were tested on the pectic media. Whenever sucrose was present, abundant moist growth, characteristic of the cultures used, was obtained. In the controls with no added carbohydrate, growth was exceedingly scanty (only nutrients of the root extract being available, and in these pectic test media only one-half of the strength of root extract ordinarily used in the Rothamsted Laboratory had been used). In the plain pectic media, growth was no better than in the controls, and there was no indication of utilisation of the pectins or calcium pectate.

It would be interesting to try the effect of the bacteria on the hemicellulose constituent of the root-hair walls, but so far it has not been possible to isolate the specific hemicellulose in anything like sufficient quantity for the preparation of media. On the other hand, there is little likelihood that the bacteria would be able to destroy it in culture, when they apparently do not do so in the root hair itself. Whether the root hair wall is destroyed at the exact point of the bacteria, or whether it is re-formed there after invasion, are points too fine to be settled by direct observation.

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* These tests were made on the agar films dried in an oven at 100° C.

† From the British Drug Houses.

‡ From the California Fruit Growers' Exchange Laboratory.

§ Prepared from the lemon pectin.

A study of the root hair infection thus leads to the following conclusions :—

(1) The bacteria do not enter mechanically injured root hair tips,

(2) they have not been shown capable of dissolving the known constituents of the root hair walls, but

(3) they do produce a secretion capable of modifying the wall in some way, as evidenced by abnormal curling of the root-hair tip either in the presence of the bacteria or of their fresh metabolic products.

It is further suggested that the bacteria attack the tip of the root hair because of some pre-existing physical difference at that point, as evidenced by its susceptibility to osmotic change.

There is curious limitation of the infection of the curled and bent root hairs. The great majority are not visibly infected. Thornton (1929) has reported 4 per cent. infected root hairs for lucerne seedlings and the various counts made in the present study have ranged from 2·4 to 5·5 per cent. There is also some limitation of the number of infections which develop into nodules. Few counts of this phase of the problem have been made, but it seems of interest to present one set of figures relating to one of the lucerne plants studied :—

Total number of hairs on 1 inch of root	1121
Number of infected hairs in above section	62
Percentage of hairs infected	5·5
Number of nodules on the plant	5
*Estimated total length of root system (inches)	5·5
Estimated total number of hairs	6165
Estimated total number of hairs infected	341

The plant was approximately 6 weeks old; its five nodules were well grown and apparently no new nodules were forming. Consequently it appears that only 1 in 68 of the original infections succeeded in producing a nodule. The other infections apparently stopped growing, many with the infection thread only part way down the root hair.

PART II.—THE INFECTION THREAD WITHIN THE NODULE.

Any attempt to explain the invasive powers of the nodule bacteria must account for their original penetration of the root hairs and also for the spread of infection through the cortical tissues of the root and, later, throughout the

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* At the time of the count bearing or having borne root hairs.

growing nodule. Many of the histological studies on root nodules have referred to the infection threads as " passing from cell to cell " and thereby extending the infection. Recently Milovidov (1926, 1928) has found that infection in the lupin is spread through active division of the host cells, but it is admitted that the lupin is exceptional in this respect and that in the majority of Leguminosæ the infection threads are active. It remains, then, to show how the infection threads cross cell walls.

As in Part I of this report, the microchemical investigation was begun by a study of the normal constituents of the parenchymatous tissue of the root cortex and of the nodule. Briefly it may be said that the methods used were the same as those described for the study of root hairs, and that the following constituents were found: cellulose, calcium pectate and probably pectose, and a hemicellulose less resistant than that of the root-hair wall (see p. 515). The hemicellulose of the parenchymatous tissue is, for instance, destroyed by heating to 300° C. in pure glycerine or boiling 1 hour in 5 per cent. KOH; is not destroyed by boiling 1 hour in 3 per cent. HCl, but is destroyed by subsequent heating 1 hour in 5 per cent. Na_2CO_3 or 0·5 per cent. KOH for ½ hour to 1 hour; is destroyed by 6 hours' boiling in 3 per cent. HCl alone, but not by 0·5 to 2 per cent. KOH 1 to 2 hours nor for 6 hours in 0·5 per cent. $(NH_4)_2C_2O_4$ it is not soluble in copper-oxide-ammonia or concentrated ammonia in the cold, but is slowly soluble in eau de Javelle in the cold. It does not interfere with the chlor-zinc-iodide test for cellulose; it is stainable by ruthenium red and therefore interferes to some extent in the study of the pectic substances in the nodule. It is stained slightly or not at all by iodine green.

In an elongated nodule of the lucerne type, for instance, the distal end contains the meristematic tissue, and the main body of the nodule, as one passes toward the proximal or basal end, is composed of progressively older tissue. Thus the tissue is graded from the young and meristematic to the old and mature, and one would expect the composition of the walls to vary accordingly. In the following discussion only the meristematic tip and parenchymatous tissue of the mature bacteroid area are discussed. The walls in question are very thin, and it is not always possible to differentiate between substances in the middle lamellæ and secondary layers of the walls.

Priestley and Tupper-Carey (1922) and Tupper-Carey and Priestley (1924) have studied the difference in composition of the meristematic and mature walls of root and stem tips and have reported that a Congo red-acid reaction reveals one striking difference. Meristematic walls, once stained by aqueous Congo red, remain red in the presence of 10 per cent. acetic acid, because of the specific isoelectric point of the protein which they contain. Mature walls, devoid of protein, are turned blue by this acid. The reaction was highly successful when applied to longitudinal sections of fresh nodule tissue; it showed distinctly red walls in the meristematic cap, grading to blue walls in the older bacteroid area. Other sections of the same nodule were held over 24 hours at 37° C. in a peptic-digest bath containing 0·3 per cent. HCl, and then tested with the Congo red-acid reaction. This time the meristematic walls also were blue, indicating that the proteolytic enzyme had removed the substance responsible for the original red staining. Moreover, the bacterial bodies which previously gave the protein reaction were much shrivelled by the peptic digestion, and their residues were thereafter blue in the Congo red-acid test.

In its pectic and hemicellulosic content also, the meristematic cap differs from the old tissue. The oxalate test for calcium pectate, for instance, shows well-formed calcium oxalate crystals in the older tissue and absolutely none in the extreme meristematic tip. But there is some form of pectic material in the tip, as evidenced by the following observations. Ruthenium red colours fresh tissue pink throughout the bacteroid and meristematic areas, indicating either hemicellulose or pectic material or both. Copper-oxide-ammonia at this stage fails to attack the tissue. Other tissue boiled 6 hours in 0·5 per cent. $(NH_4)_2C_2O_4$ shows older parts of the tissue still pink in ruthenium red, but the meristematic tip very pale or colourless. Copper-oxide-ammonia at this stage violently attacks the meristematic tip and dissolves its cell walls, while causing only collapse in the older tissues. These effects indicate pectic material throughout the tissue but little or no hemicellulose in the extreme meristematic tip. And since the pectic material in the tip fails to give the calcium pectate test, it may be assumed to be pectose or pectin.

Solubility and staining reactions showed the presence of cellulose in both young and old walls. The colour tests were particularly interesting because they revealed certain clustered patches devoid of cellulose. Fig. 5 is a photomicrograph of cells in the bacteroid area of a lucerne nodule stained with Schultze's reagent. Similar pits were found in the cells of the pea and clover nodules and the normal parenchyma of their root cortices as well. The patches were first seen in tissue stained with either Schultze's or the hydrocellulose test reagents (best in tissue previously treated with alkali) but are also visible in fresh tissue deeply stained with methylene blue or Congo red. One's first impression on seeing such preparations is that the patches are actual perforations in the walls, perhaps channels of the plasmodesmen.

Gardiner (1897, 1901) who studied plasmodesmen, found them in parenchyma tissue and in his 1901 paper made the following suggestive remark: "Parenchyma cells of the pith, if mounted in weak iodine containing about 10 per cent. sulphuric acid, show the cellulose walls blue excepting where the closing membrane is stained yellow." That there is actually a closing membrane across the patches in the nodule cell walls seems probable from the following observation. Some tissue boiled 1 hour in 3 per cent. HCl and stained with ruthenium red, showed very thin and delicate cell walls of the bacteroid-tissue, coloured uniformly red, apparently only the middle lamellæ being stained. Schultze's reagent, diffused under the cover-glass, revealed doubleness of the walls, which appeared much thicker than when stained with ruthenium red alone. Also the characteristic white patches appeared *in the thick blue layers of the walls.* Some pieces of tissue observed on edge showed a definitely pitted effect. And so, while the patches are evidently similar to those seen by Gardiner in the walls of parenchyma, they should rather be interpreted as pits in the secondary layers of the walls and not necessarily protoplasmic connections, as the plasmodesmen are now conceived to be. They may, of course, be the remains of the original plasmodesmen, bridged over by the middle lamellæ but with the perforations of the secondary layers of the walls enlarged and distorted by the stresses of growth. However that may be, the presence of these differentiated patches in the walls is most significant. They vary in size and shape, as may be seen from the photograph. On tissue stained with Schultze's reagent, the diameters ranged from 2·5 to 8 micra; this tissue was, of course, somewhat swollen by the reagent, so that the measurements are not to be taken as exact, but they indicate that the pits are of sufficient size to admit small infection threads. This is not a new suggestion. A search of the literature showed that Vuillemin (1888) also found the patches not stainable by chlor-zinc-iodide. He described them as "*fenêtres*" and ingeniously suggested that, thanks to them, the walls are readily permeable yet maintain their structure and strength.

The nature of the infection thread must now be considered in order that its behaviour as it crosses a cell wall may be understood. This is an old and much debated problem in the cytology of the nodule. It has been known since the early descriptions by Eriksson (1873) and Ward (1887) that the infection thread spreads out into a funnel-shaped enlargement as it crosses the wall. Ward (1888) attached no importance to the enlargement, remarking that the expansion of the cell wall after the passage of the infection thread would account for such an experience. Prazmowski (1890) described the enlargement as a passive spreading of the thread in the intercellular space, formed by the splitting apart of the middle lamella under the boring action of the infection thread. On the nature of the infection thread itself, opinion is divided between two opposing views, namely, (1) that the thread is a zooglœal strand imbedding the bacteria, or (2) that it is a definite tube in which the bacteria lie imbedded in their own slime. Several of the early investigators, Eriksson (1873), Ward (1887), Vuillemin (1888), Koch (1890), and Laurent (1891) reported the outer sheath of the infection thread to contain cellulose. Moeller (1892) and Schneider (1892) granted that such a membrane is present but held it to be a deposit by the host cell to wall off infection. Kny (1879), Prillieux (1879 and 1890), and Zukal (1897) found no membrane and therefore considered the threads to be plasmodia. Dawson (1900) failed to confirm the early reports of a cellulose membrane but recognised that the infection thread is tube-like and bounded by a definite sheath. Peirce (1902) and Fred (1909) referred to the threads as zooglœal strands, and Burrill and Hansen (1917) remarked their "solid hypha-like structure bearing remarkable resemblance at times to a tube."

Anyone who studies the infection threads carefully must be impressed by their great variety of form, yet definiteness of boundary, and with the sharp contrast in refractility between them and the protoplasm of the host cells. This is particularly evident in the infection threads in the root hairs, examined fresh in a water mount. Often in such mounts some infected hairs will be found mechanically broken, and then one may see the infection thread projecting from the open end, as rigid and clear as a glass rod. Or perhaps the lateral walls of the hairs will be broken away and the infection thread, still intact, may be seen holding the broken hair in place. A gentle tap on the cover-glass will prove how stiff and resistant is such an infection thread. It is hard to imagine a zooglœal strand behaving so. Moreover, in prepared slides it is common to see an outer layer or sheath which is definitely stained by the same dyes that affect the cell walls of the plant. Sometimes, however, the sheath may be very poorly developed or not even present. These naked threads are usually seen in very young infection spots or in cells apparently newly invaded.

A microchemical study of the sheaths of infection threads was made in order to discover their probable origin. They were first tested for cellulose, which was considered to be present on the basis of the following observations :—

1. Sheaths survive—

   (*a*) Boiling 6 hours in 3 per cent. HCl.

(*b*) Boiling 1 hour in 3 per cent. HCl or $H_2C_2O_4$, followed by 1 hour in 5 per cent. KOH.

(*c*) Heating to 300° C. in glycerine (alone or after pre-treatment with 3 per cent. boiling HCl 1 hour and 5 per cent. boiling KOH 1 hour).

(*d*) Boiling 0·5 per cent. $(NH_4)_2C_2O_4$ 1 to 6 hours.

(*e*) Heating to 180° C. in 50 per cent. KOH.

2. Sheaths in tissue treated as in (*a*), (*b*) and (*c*) above instantly soluble in copper-oxide-ammonia.
3. Sheaths in tissue after any of the above treatments show positive staining with Schultze's or the hydrocellulose test reagents. Fresh tissue also gives a positive test for cellulose but not so deep a colour.

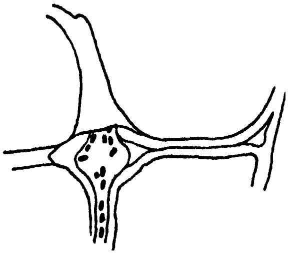

Fig. 1.—A camera lucida drawing of the sheath of an infection thread as it approaches the cell wall.

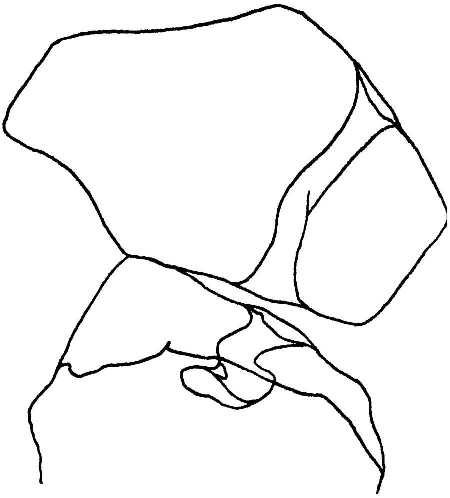

Fig. 2.—A camera lucida drawing showing more clearly the separation of the host cells and the absence of sheath across the middle lamella.

The colour tests for cellulose revealed an interesting and significant fact about the infection thread. Tissue treated to remove pectic and hemicellulosic materials is necessarily fragile and easily macerated, owing to loss of its "binding" materials. The cellulose tests on such material produce a certain swelling which tends to separate the cells and shows that *there is no sheath around the infection thread as it crosses the middle lamella.* Text-figs. 1 and 2 are camera lucida sketches of such pieces of infection thread. In one test on tissue previously treated for 10 days in eau de Javelle in the cold and boiled 1 hour in 0·5 per cent. $(NH_4)_2C_2O_4$, the cells actually broke apart and floated away in the currents under the cover-glass. Several were observed in which the sheaths of the infection threads appeared as complete tunnels through the cells. Such observations provide strong evidence that the sheath is a deposit of the individual plant cell and not of the bacterial strand. Also the sheath is obviously attached to the plant cell wall through the "funnel-shaped enlargement"; it is of the identical shade of blue in the cellulose-tested material and merges imperceptibly into the lateral walls of the cell. The expansion of the slime thread lies entirely within the middle lamella, as Prazmowski pointed out.

The sheath also contains other constituents which relate it to the plant wall. Material extracted for 10 days in copper-oxide-ammonia and no longer giving the cellulose reaction, still contains the sheaths of its infection threads. These sheaths are stainable with ruthenium red and thus may be either pectic or hemicellulosic. That they are not composed of calcium pectate is shown by negative oxalate tests. They do contain the hemicellulose of the plant cell wall as shown by their survival of 6 hours extraction with 0·5 per cent. $(NH_4)_2C_2O_4$; after which they can still be stained by ruthenium red but not by iodine green. They respond also to other properties of the hemicellulose noted earlier in this paper in connection with the study of the walls of the bacteroid cells. The presence of the hemicellulose in the sheaths interferes with the detection of such pectic substances as pectose or pectin.

There is one other line of evidence that may be cited in favour of the host plant origin of the infection-thread sheaths. In material which has been heated to 300° C. in pure glycerine, one often finds diamond-shaped pieces of cellulosic infection-thread sheath attached to some of the cell walls. This indicates that the sheaths form first at the cell walls, and would suggest that they originate at the cell walls and not along the gummy matrix of the thread, which extends through the cell as well as across its boundaries.

SUMMARY AND CONCLUSIONS.

Part I.—The Infection of Root Hairs.

(1) It is statistically proved that infection of the root hairs is not a mere invasion of mechanically injured or broken root hairs. The presence of the bacteria, even of strains belonging to foreign inoculation-groups, causes a significant increase in the number of curled and bent hairs.

(2) The nodule bacteria invariably attack the tip of a root hair, a fact which may be related to some pre-existing physical difference at that point.

(3) The bacteria produce a secretion capable of modifying the wall, as evidenced by the abnormal curling of the root-hair tip. This secretion is separable from the cells by filtration and is not specific for the plants of the cross-inoculation group to which the bacteria belong.

(4) The bacteria in culture could not be shown to attack cellulose, pectin or calcium pectate. Curled tips of root hairs, whether or not infected, contain the same constituents as normal hairs. These constituents are cellulose, calcium pectate and probably pectose, and a very resistant hemicellulose.

Part II.—The Infection Thread within the Nodule.

(5) The cell walls of the nodule contain cellulose, a hemicellulose, calcium pectate in the mature parts, and pectose at least in the meristematic tip. Walls of the tip also give a protein reaction.

(6) There are numerous pits perforating the secondary layers of the walls, but the middle lamellæ appear to be continuous. These pits are of sufficient size to admit infection threads.

(7) The infection thread is surrounded by a definite sheath consisting of cellulose and hemicellulose; calcium pectate is absent and the presence of other pectic materials has not been confirmed.

(8) The sheath does not cross the middle lamella, and is probably a deposit of the individual plant cell.

(9) It is suggested that the bacterial zoogloea crosses a cell wall by way of the pits and is later covered by the sheath, a product of host cell activity.

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EXPLANATION OF FIGURES.

Plate 26.

Fig. 1.—A photomicrograph of root hairs of a pea plant grown in the presence of lucerne bacteria.

Fig. 2.—A photomicrograph of normal root hairs of a clover plant.

Fig. 3.—A photomicrograph of root hairs of a clover plant grown in the presence of a filtrate of lucerne bacteria.

Fig. 4.—A photomicrograph of root hairs of a lucerne plant grown in the presence of a filtrate of clover bacteria.

Fig. 5.—A photomicrograph of the walls in the bacteroid area of a lucerne nodule stained with Schultze's reagent. The pits in the secondary layers of the walls are visible as white patches.

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THE *AZOTOBACTER* TEST OF SOIL FERTILITY APPLIED TO THE CLASSICAL FIELDS AT ROTHAMSTED.

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(With Plate IV.)

The sensitiveness of the *Azotobacter* group of organisms to soil acidity and to lack of available phosphate has been long recognised and has been the basis of microbiological tests of both these soil conditions, devised especially by Christensen and Niklas. Such methods have lately (10, 12) been criticised by Winogradsky. The *Azotobacter* cells are often placed under semi-anaerobic conditions in a liquid medium, where many may perish from lack of oxygen or from competition with anaerobic acid-producers such as *Clostridium*. The author (5) in fact found that the Christensen-Niklas method sometimes failed to detect the presence of *Azotobacter* in the soil and consequently, as a test of lime or phosphate deficiency led to misleading results. In 1926–7 a new *Azotobacter* test was developed by Winogradsky and the author (1, 2), who termed it *la méthode des plaques moulées*. It has since been applied to French soils by Guittonneau (3), to Russian soils by Krjutshkova (4), to Polish soils by the author (5, 6, 7, 8), and to some American soils by Sackett and Stewart (13). The method was successful, although slight modifications were needed for different soil types.

TECHNIQUE.

In principle the kneaded-plate method consists in observing the development of *Azotobacter* colonies upon the surface of the soil itself, suitably moistened and kneaded. About 150 gm. of the fresh sifted soil are mixed with 1 per cent. mannitol or organic acid salts in the case of light soil, or with 5 per cent. starch in the case of heavy soil. In light soils lacking in colloids 10–20 per cent. of sterilised kaolin is added to increase the plasticity. The mixture is then divided into four portions. The first portion is moistened with distilled water, the second with a solution containing 0·67 gm. Na_2HPO_4 + 0·33 gm. NaH_2PO_4 per litre, giving a 0·1 per cent. solution of phosphate having a reaction of pH 7·0. (In rare instances a stronger phosphate solution is needed.) The third portion is mixed with $CaCO_3$ (at least 2 per cent.) and moistened with distilled water, and the fourth portion is mixed with $CaCO_3$ and moistened with the phosphate solution. This gives four treatments as follows:

0 = control,
+ P = phosphate added,
+ Ca = calcium carbonate added,
+ P + Ca = calcium carbonate and phosphate added.

Each portion is moistened to the point of saturation and is then kneaded with a pestle and mortar until a fine paste is obtained. Petri dishes of 3–4 cm. internal diameter are filled with the pastes and the surfaces smoothed by means of a glass slide moistened with sterile water. The plates are left uncovered and incubated in a moist chamber at 30° C. After 40 hours to 3 days the *Azotobacter* growth becomes visible in the form of milky drops or compact white growth. In the early stages of incubation such colonies consist of nearly pure cultures of *Azotobacter*. A quantitative estimation of the growth can be made by counting the colonies on a square centimetre of surface, using a lens. When a good growth of *Azotobacter* takes place on all the plates it is inferred that the soil contains sufficient lime and available phosphate for the needs of the organisms. A shortage of available phosphate or lime in the original soil is revealed by *Azotobacter* growth being visible only on the portions to which the deficient constituent has been supplied (see Plate IV).

It is sometimes found that no *Azotobacter* growth takes place with any of the treatments. This may mean that *Azotobacter* is absent from the original soil or that it has been suppressed by competition with other soil organisms. This suppression is especially liable to occur in soils rich in available nitrogen, which encourages the competition. On plates of such soils the *Azotobacter* is often replaced by an organism producing a vitreous sticky growth (the *Bacille gommeux* of Winogradsky) which appears after 30–48 hours' incubation and is followed by a growth of moulds. The test for lime and available phosphate by the above method will fail where *Azotobacter* is absent or suppressed in the original soil, so that abundance of available nitrogen may conceal the presence or absence of phosphate or lime. For such soils, the method may be modified by supplying *Azotobacter* from a fresh culture which may be grown either on silica jelly plates or in sterilised soil to which a carbohydrate has been added. The addition of *Azotobacter* to the sample is a valid proceeding, because the *plaque moulée* method aims at testing whether the soil is suitable for the growth of *Azotobacter* and not whether it was originally present.

Fig. 1

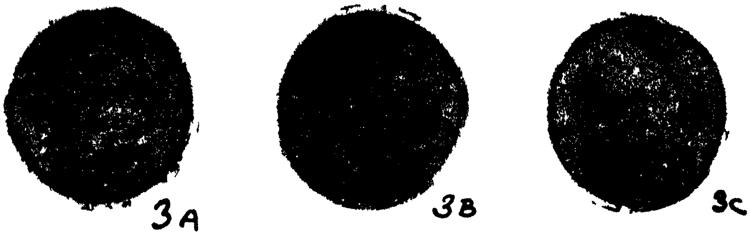


Fig. 2

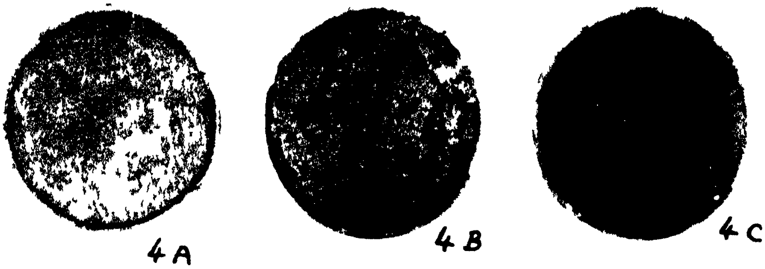
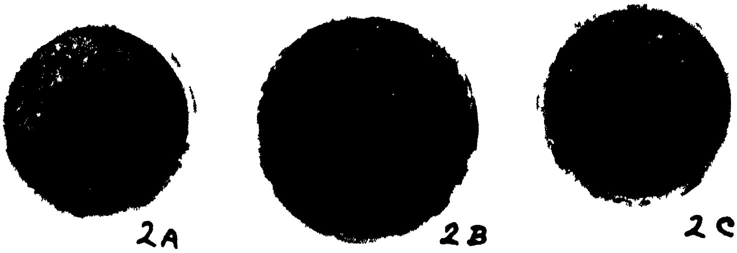


Fig. 3



Kneaded plates (*plaques moulées*) made from selected Hoos Field soils: 4 days' incubation at 30° C. Approximately two-thirds natural size

Fig. 1. A–C. Plot 3-A, receiving no phosphate, soil reaction *p*H 7·2
Fig. 2. A–C. Plot 4-A, receiving phosphate, soil reaction *p*H 5·5.
Fig. 3. A–C. Plot 2-A, receiving phosphate, soil reaction *p*H 7·9.

All these plots received 43 lb. of nitrogen per acre as ammonium sulphate. With each soil, plate A was given phosphate, plate B calcium carbonate, and plate C neither lime nor phosphate.

The density of the *Azotobacter* population in the original soil may be estimated by the method of silica jelly plates, devised by Winogradsky (10). Silica jelly is prepared by adding 100 c.c. potassium or sodium silicate solution of specific gravity 1·06–1·08 to 100 c.c. HCl specific gravity 1·10[1] in a beaker. The solution is well mixed and poured into petri dishes 20 cm. in diameter. In about 48 hours the gel will have set and the HCl is washed out of it with flowing tap water, the washing being finished off with boiled distilled water. 1 gm. of mannitol or 0·5 gm. of the more selective calcium lactate is sprinkled over the surface of each plate, which is then watered with 10 c.c. of the following solution:

KH_2PO_4	0·5 gm.
$MgSO_4$	0·3 ,,
NaCl	0·3 ,,
$FeSO_4$	0·01 ,,
$MnSO_4$	Trace
Water	100 c.c.

The *p*H adjusted to 7·0–7·2 with 2 per cent. KOH.

Where mannitol is used, 0·5 gm. $CaCO_3$ should also be added to each plate. The supernatant solution on the plates is evaporated at 40° C., and 50 mg. (dry weight) of the fresh sifted soil is evenly distributed over the surface of the jelly. The amount of soil may be increased to 1 gm. where a thin population of *Azotobacter* is expected. The plates are incubated for 48 hours at 30° C. and the *Azotobacter* colonies are counted. The advantages of this method over the dilution plating technique are elsewhere discussed (5).

APPLICATION OF THE *AZOTOBACTER* TEST TO THE BROADBALK WHEAT PLOTS.

The classical fields at Rothamsted and Woburn afford a unique opportunity for testing methods of microbiological soil analysis, since on them the results can be correlated with manurial treatment and with yield data extending over a longer period than is obtainable elsewhere. The methods described above were therefore applied to the wheat plots on Broadbalk, to the portion of Broadbalk left as wilderness, to the barley plots on Hoos Field, to the mangold plots on Barnfield and to the rotation plots on Agdell field. For comparison with these heavy soil fields, some tests were also applied to sandy soil plots under permanent wheat and barley at Woburn, and to an acid loamy soil from a pasture in Cheshire. The samples were taken from surface soil, to a depth of about 5 in., each sample being a composite of 3 or 4 cores. The soils were passed through a 1 mm. sieve. The tests were applied one day after sampling, with the exception of samples collected in 1930 which were sent to the Soil Research Laboratory in Poznań University, Poland, for the examination, which took place some weeks after sampling. In making the kneaded plates, mannitol was added to the soils, as it was found to be more satisfactory than starch. In addition to the *Azotobacter* tests, the water-soluble phosphate was estimated by the field method of Spurway, as developed by Terlikowski and Królikowski (9), and in some cases the *p*H was determined.

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[1] Made by adding 400 c.c. distilled water to 600 c.c. HCl specific gravity 1·19.

Samples from Broadbalk were taken on 2nd August, 1930, on 11th September, 1931 and on 13–14th October, 1931. The results obtained are shown in Tables I–III. The best conditions for *Azotobacter* growth on all three occasions were found in Plot 5, which received complete minerals but no nitrogen. On this plot very numerous *Azotobacter* colonies developed on the kneaded plates; their number was not increased by the addition of either phosphate or lime, indicating no shortage of either constituent. The control Plot 3 gave *Azotobacter* growth on the kneaded plates only where phosphate was supplied, indicating a deficiency in this element. The dunged Plot 2 B showed no evidence of phosphate deficiency but the number of *Azotobacter* colonies on the kneaded plates was much reduced, and was even lower than the control plot when the latter's phosphate deficiency was made up.

The most striking feature of Broadbalk samples, however, was the repression of *Azotobacter* in plots receiving mineral nitrogen. This effect of nitrogen can be well studied in Plots 6, 7, 8, 9 and 16, where different nitrogen dressings have been given to a uniform dressing of minerals which Plot 5 shows to afford excellent conditions for *Azotobacter* growth. It can also be seen in the samples from Plots 10 to 14, where the nitrogen supply is kept constant and the minerals varied. *Azotobacter* colonies were absent or scarce on uninoculated kneaded plates made from all these plots which receive mineral nitrogen, and this scarcity was unaffected by the addition of phosphate or lime to the plates. It was clearly related to the paucity of *Azotobacter* cells in the original soil, as is shown by the counts made on silica jelly plates, which show an inverse relationship between the *Azotobacter* numbers and the nitrogen dressing.

The reduction of *Azotobacter* population in the soil of these plots can be explained as being due to competition with other organisms which thrive on the nitrogen supplied. It has been shown (2) that if soil rich in *Azotobacter* is added to a medium containing mannitol and varying doses of nitrate, the development of *Azotobacter* becomes weaker with increasing nitrate until it stops completely when the C : N ratio reaches 100C : 0·4N. At this point the *Bacille gommeux*, moulds, and other organisms replace it. It is evident therefore that *Azotobacter* can compete for the energy supply only under conditions of nitrogen shortage. It was

Table I. *Broadbalk.*

Soil samples taken on 15th July, 1930, examined 2nd August, 1930. Manures applied: Dung, September; Minerals and Rape cake, October; Ammonium sulphate, October and March; Nitrate, March (and on Plot 16, half in April).

Soil sample no.	Plot	Plot treatment	Water soluble P_2O_5 mg. per kg.	Uninoculated kneaded plates. Number of *Azotobacter* colonies on 1 cm.^{2} 0	+P
1	2B	Dung, 14 tons per acre	>15	50	50
2	3	Control	6–10	0	50–100
3	5	Complete minerals*	>15	80–100	80–100
4	8	129 lb. N per acre as amm. sulph. + complete minerals	>15	1–2	1–2
5	16	86 lb. N per acre as nitrate of soda + complete minerals	>15	10	10

* Complete minerals – 3½ cwt. superphosphate 200 lb. sulph. potash, 100 lb. sulph. soda, 100 lb. sulph. magnesia.

Table II. *Broadbalk.*

Soil samples taken and tested on 11th September, 1931. Manures applied: Dung, October; Minerals and Rape cake, October; Ammonium sulphate, October and March; Nitrate, March (and on Plot 16, half in May).

Soil sample no.	Plot	Plot treatment	Yields in 1930. Grain, bushels per acre	Water soluble P_2O_5 mg. per kg.	Number of *Azotobacter* colonies from 1 gm. soil on duplicate silica plates	Uninoculated kneaded plates. Number of *Azotobacter* colonies on 1 cm.^{2} 0	+P	+Ca	+P+Ca
6	2B	Dung, 14 tons per acre	34	11–15	(*a*) 1479 (*b*) 945	10–15	15	—	15
7	3	Control	12	0–5	(*a*) 592 (*b*) 936	0	20	—	20–25
8	5	Complete minerals	14	11–15	(*a*) 8048 (*b*) 8752	50–70	50–70	—	—
9	8	As 5 + 129 lb. N per acre as amm. sulph.	35	11–15	(*a*) 31 (*b*) 40	0	0	2–3	2–3
10	16	As 5 + 86 lb. N per acre as nitrate	31	11–15	(*a*) 276 (*b*) 326	10–15	10–15	—	10–15
11	19	As 5 + rape cake	22	11–15	(*a*) 155 (*b*) 209	1–2	3–4	3–5	3–5

Note. In this and the following tables a dash, —, means that the particular test was not made, a nought, 0, that the test was negative.

Table III. *Broadbalk.*

Soil samples taken on 13–14th October, 1931. For times of manure application see Table II.

Soil sample no.	Plot	Plot treatment	Yields in 1930. Grain, bushels per acre	pH	Water soluble P_2O_5 mg. per kg.	Number of *Azotobacter* colonies from 1 gm. soil on duplicate silica plates	Kneaded plates							Remarks
							Uninoculated soil. Number of *Azotobacter* colonies on 1 cm.²					Soil inoculated with *Azotobacter* culture		
							0	+P	+Ca	+P+Ca	+K	0	+P	
12	2B	Dung, 14 tons per acre	34	—	ca. 15	(*a*) 118	20	20	—	—	—	—	—	—
13	3	Control	12	8·0	ca. 5	(*a*) 844	0	30	0	—	—	—	—	—
14	5	Complete minerals	14	—	>15	(*a*) 2268 (*b*) 1842	30–40	30–40	—	—	30–40	+++	—	—
15	6	As 5+43 lb. N per acre as amm. sulph.	22	7·1	>15	(*a*) 852 (*b*) 1113	15–20	15–20	15–20	15–20	15–20	+++	+++	*Azotobacter* well developed but mixed with *Bac. gommeux* and moulds
16	7	As 5+86 lb. N per acre as amm. sulph.	31	7·4	>15	(*a*) 253 (*b*) 253	0	0	0	0	0	+++	+++	*Azotobacter* present, but formed no colonies. Thick growth of *Bac. gommeux* and moulds
17	8	As 5+129 lb. N per acre as amm. sulph.	35	6·5	>15	(*a*) 27 (*b*) 0	0	0	0	0	0	+++	+++	Thick growth of *Bac. gommeux* and moulds. No *Azotobacter* development
18	9	As 5+43 lb. N per acre as nitrate of soda	25	7·4	11–15	(*a*) 1862 (*b*) 1324	10–15	10–15	—	—	—	+++	+++	—
19	10	86 lb. N per acre as amm. sulph. No minerals	19	—	6–10	(*a*) 195 (*b*) 322	0	5–10	0	+K+P+Ca 5–10	0	0	++	—
20	11	As 10+P	21	—	>15	(*a*) 187 (*b*) 252	3–5	3–5	3–5	+K+P+Ca 3–5	3–5	—	—	—
21	12	As 10+P+Na	28	—	>15	(*a*) 54	5–10	5–10	5–10	+K+P+Ca 5–10	5–10	—	—	—
22	13	As 10+P+K	30	—	>15	(*a*) 393 (*b*) 381						—	—	Counts not possible. *Azotobacter* mixed with *Bac. gommeux* and moulds
23	14	As 10+P+Mg	27	—	>15	(*a*) 75 (*b*) 100	8	6	6	—	6	—	—	—
24	15	As 5+86 lb. N per acre as amm. sulph. All applied in Oct.	28	—	>15	—	0	0	0	—	—	+++	+++	Thick growth of *Bac. gommeux*
25	16	As 5+86 lb. N per acre as nitrate	31	—	>15	(*a*) 130 (*b*) 109	2–3	2–3	—	—	—	+++	—	—
26	17	Complete minerals Oct. 1930. Amm. sulph. 9th Oct. 1931	29	—	11–15	—	40	40	—	—	—	—	—	—
27	18	Amm. sulph. Oct. 1930 and March, 1931. Complete minerals 9th Oct. 1931	14	—	11–15	—	15	15	—	—	—	—	—	—
28	19	Rape cake equivalent to 86 lb. N	22	—	11–15	—	0	15	1	—	—	—	—	Moulds on all plates
29	20	43 lb. N as amm. sulph. Minerals, but no P	19	—	11–15	—	0	2–3	—	—	—	—	—	—

observed that the samples from Broadbalk Plots 6, 7 and 8 showed a progressive increase in spore-forming bacilli and moulds as well as a progressive reduction in *Azotobacter* colonies, corresponding with the increase in nitrogen dressing. The standard kneaded-plate method thus fails to detect phosphate or lime deficiency in the presence of such mineral nitrogen dressings as are given to Broadbalk, owing to the reduced numbers or lessened viability of *Azotobacter* in the original soil. Kneaded plates inoculated with *Azotobacter* were therefore made with some of the soil samples. When this was done a normal development of *Azotobacter* colonies was always found where the necessary phosphate and lime were present.

The kneaded-plate method was also applied to that portion of Broadbalk which has been left as wilderness since 1882. Half of this piece is now woodland and half is kept in grass and weeds. The test (Table IV) showed phosphate deficiency in both portions of the wilderness, but no acidity. The woodland portion contained a far smaller population of *Azotobacter* than the herbaceous half, but even the latter contained fewer *Azotobacter* than the unmanured Plot 3 in the cultivated portion of the field.

Table IV. *Broadbalk Wilderness since* 1882.

Soil samples taken on 11–12th November, 1931.

Soil sample no.		Water soluble P_2O_5 mg. per kg.	Number of *Azotobacter* colonies from 1 gm. of soil on silica plates	Kneaded plates. Number of *Azotobacter* colonies on 1 cm.^{2}					
				Uninoculated				Inoculated	
				0	+P	+Ca	+PCa	0	+P
30	No trees	*ca.* 5	(*a*) 317 (*b*) 358	0	2–3	0	—	0	++
31	Wood	0–5	(*a*) 139 (*b*) 100	0	2–3	0	—	0	++

THE HOOS FIELD BARLEY PLOTS.

Samples from Hoos Field were taken 15th July, 1930, 16th September, and 2nd November, 1931 (see Tables V, VI, VII). Repetition of the same mineral dressings with various forms of nitrogen makes this field particularly suitable to the application of microbiological analysis. As with Broadbalk, the best *Azotobacter* growth on kneaded plates and the highest number of colonies on silica plates were given by the plot receiving complete minerals but no nitrogen, while the control Plot 1-O gave a lower colony count on silica plates and showed phosphate deficiency on the kneaded plates. The dung Plot 7-2 again gave rather fewer colonies on the kneaded plates than did the no-nitrogen plots where phosphate was present, although the silica plate counts made on 16th September from this plot gave high figures. Plot 7-1, which received dung from 1852 to 1871 and has since been unmanured, showed a phosphate deficiency like the unmanured Plot 1-O, but gave a somewhat higher silica plate count than did the latter (Table VI).

The plots receiving nitrate, ammonia or rape cake showed a depression in the *Azotobacter* count on silica plates and a corresponding failure or weak development of colonies on the kneaded plates. In the case of

Table V. *Hoos Field.*

(*a*) Soil samples taken 15th July, tested 2nd August, 1930. Manures applied in March.

Soil sample no.	Plots	Plot treatment	Water soluble P_2O_5 mg. per kg.	Uninoculated kneaded plates 0	+P
32	1-O	Control	0–5	0	Numerous
33	4-O	Complete minerals	>15	>50	>50
34	4-A	As 4-O + 43 lb. N per acre as amm. sulph.	>15	0	0
35	4-AA	As 4-O + 43 lb. N per acre as sodium nitrate	11–15	0	0
36	7-1	Dung in 1852–71, since then unmanured	6–10	0	50–100

Table VI. *Hoos Field.*

(*b*) Soil samples taken on 16th September, 1931. Manures applied in March.

Soil sample no.	Plots	Plot treatment	Yields in 1930. Grain, bushels per acre	Water soluble P_2O_5 mg. per kg.	Number of *Azotobacter* colonies from 1 gm. soil on silica plates	Uninoculated kneaded plates 0	+P	+Ca	+P+Ca
37	1-O	Control	14	5–10	(*a*) 1368 (*b*) 1245	0	50	—	50
38	4-O	Complete minerals	20	11–15	(*a*) 3244 (*b*) 2062	100	100	—	100
39	4-A	As 4-O + 43 lb. N per acre as amm. sulph.	41	>15	(*a*) 100 (*b*) 36	0	0	1–5	1–5
40	4-AA	As 4-O + 43 lb. N as nitrate of soda	39	>15	(*a*) 554 (*b*) 558	50	50	—	—
41	4-C	As 4-O + rape cake	39	>15	(*a*) 117 (*b*) 190	0	0	0–1	0–1
42	7-1	Dung only in 1852–71, since then unmanured	24	0–5	(*a*) 1859 (*b*) 1739	0	35–40	—	—
43	7-2	Dung every year	46	>15	(*a*) 2098 (*b*) 2007	15–20	20	—	—

Plot 4-A this seemed to be associated with acidity. In spite of the depression in *Azotobacter* population in the soils with nitrogen manuring, the uninoculated kneaded plates gave a correct diagnosis of phosphate and lime deficiency more often than was the case on Broadbalk (see soil samples 40, 48, 49, 50, 51, 53, 55, 56 and 57). This can perhaps be attributed to the lower nitrogen dressings given to most plots on Hoos Field. Tests with inoculated kneaded plates were made from Plots 1-AA, 3-AA, 3-C and 4-C, *i.e.* where the uninoculated kneaded plates failed to show growth (samples taken 2nd November (Table VII)). These inoculated plates gave good growth where phosphate was present in the original soil or supplied in the test.

Table VII. *Hoos Field.*

(c) Soil samples taken on 2nd November, 1931.

Soil sample no.	Plots	Plot treatment	Yields in 1930	*p*H	Water soluble P_2O_5 mg. per kg.	Number of *Azotobacter* colonies from 1 gm. soil on silica plates	Kneaded plates: Uninoculated 0	+P	+Ca	+P+Ca	Inoculated 0	+P
44	1-O	Control	14	7·6	6–10	—	0	25	> 0	—	—	—
45	2-O	P	20	—	11–15	—	50	50	50	—	—	—
46	3-O	Na, K, Mg	15	—	0–5	—	(30–40)*	30–40	—	—	—	—
47	4-O	Complete minerals	20	—	11–15	—	40–50	40–50	—	—	—	—
48	1-A	43 lb. N per acre as amm. sulph.	25	8·0	0–5	—	0	15	0	—	—	—
49	2-A	As 1-A+P	37	7·9	11–15	—	30	30	30	—	—	—
50	3-A	As 1-A+Na, K, Mg	27	7·2	0–5	—	0	20–25	0	—	—	—
51	4-A	As 1-A+complete minerals	41	5·5	6–10	—	0	0	20	—	—	—
52	1-AA	43 lb. N per acre as nitrate	25	7·8	6–10	(*a*) 462 (*b*) 400	0	0	—	0	0	+++
53	2-AA	As 1-AA+P	40	7·4	>15	—	15	15	—	—	—	—
54	3-AA	As 1-AA+Na, K, Mg	26	7·1	0–5	(*a*) 250	0	0	—	—	0	+++
55	4-AA	As 1-AA+complete minerals	39	7·1	>15	—	25	25	—	—	—	—
56	1-C	49 lb. N per acre as rape cake	37	7·2	6–10	—	0	20–25	0	—	—	—
57	2-C	As 1-C+P	39	7·1	11–15	—	20	20	—	—	—	—
58	3-C	As 1-C+Na, K, Mg	35	6·0	0–5	(*a*) 16	0	0	0	—	+	+++
59	4-C	As 1-C+complete minerals	39	7·2	11–15	(*a*) 116	0	0	0	—	+++	+++
60	6-1	Unmanured since 1852	15	—	0–5	—	0	20	—	—	—	—
61	6-2	Coal ashes	16	—	6–10	—	0	20	—	—	—	—
62	7-1	Dung in 1852–71, since then unmanured	24	—	6–10	—	0	25	—	—	—	—
63	7-2	Dung every year	46	6·8	11–15	—	20	20	—	—	—	—

* *Azotobacter* growth very weak on kneaded plate 0, sample 46, but very strong on plate +P of this sample.

THE BARNFIELD MANGOLD PLOTS.

Samples from some of the Barnfield plots were taken and tested in 1930 and 1931 (Table VIII). The kneaded plates showed no phosphate or lime deficiency, but the *Azotobacter* growth on them was weak. It is possible that the ploughing in of the mangold leaves on these plots may have reduced the *Azotobacter* population, by supplying nitrogenous energy material to organisms competing with the *Azotobacter* in the soil.

Table VIII. *Barnfield.*

(*a*) Soil samples taken 15th July, tested 2nd August, 1930. Dung applied November; Minerals and Rape cake, May; Ammonia and Nitrate, May and July.

Soil sample no.	Plots	Plot treatment	Yields in 1930. Mangolds, tons per acre	pH	Water soluble P_2O_5 mg. per kg.	Number of *Azotobacter* colonies from 1 gm. soil on silica plates	Uninoculated kneaded plates. Number of *Azotobacter* colonies on 1 cm.^{2}			
							0	+P	+Ca	+P+Ca
64	1-O	Dung only	17·8	—	11–15	—	15	15	—	—
65	4-O	Minerals	4·8	—	>15	—	25	25	—	—
66	4-A	86 lb. N per acre as sulph. amm. + minerals	14·8	—	>15	—	20	20	—	—
67	4-N	86 lb. N per acre as nitrate + minerals	17·8	—	>15	—	20	20	—	—
68	8-O	Control	3·5	—	>15	—	10	10	—	—

(*b*) Samples taken 7th September, 1931. Dung applied November; Minerals and Rape cake, April; Ammonia and Nitrate, April and July.

Soil sample no.	Plots	Plot treatment	Yields in 1930. Mangolds, tons per acre	pH	Water soluble P_2O_5 mg. per kg.	Number of *Azotobacter* colonies from 1 gm. soil on silica plates	0	+P	+Ca	+P+Ca
69	1-O	Dung	17·8	7·6	>15	—	10	15–20	—	—
70	4-O	Minerals	4·8	—	>15	(*a*) 300 (*b*) 214	20	20	—	—
71	4-A	86 lb. N per acre as amm. sulph. + minerals	14·8	7·2	>15	—	5–10	10	10	10
72	4-N	86 lb. N per acre as nitrate + minerals	17·8	—	>15	(*a*) 712 (*b*) 744	15–20	15–20	—	15–20
73	4-C	184 lb. N as rape cake + minerals	21·1	—	>15	—	10	10	—	—
74	8-O	Control	3·5	8·0	11–15	—	10	10	—	—

THE AGDELL ROTATION PLOTS.

Agdell field affords an opportunity of studying the effect of long continued manurial treatment under rotation cropping. Plots 1 and 2 (Table IX), which receive sulphate of ammonia, are very acid and show no *Azotobacter* either by the kneaded-plate method or in counts made on silica plates. When these soils were inoculated with *Azotobacter*, however, the kneaded-plate test was successful in indicating lack of both phosphate and lime. Plots 3 and 4 receiving minerals with the swedes, and the control Plots 5 and 6, all show a phosphate deficiency by the kneaded-plate test. The silica plate gave more *Azotobacter* colonies from Plots 3

Table IX. *Agdell Field.*

Soil samples taken 18th November, 1931. Manures applied 1928.

Soil sample no.	Plots	Plot treatment and rotation		*p*H	Water soluble P_2O_5 mg. per kg.	Number of *Azotobacter* colonies from 1 gm. soil on silica plates	Kneaded plates. Number of *Azotobacter* colonies on 1 cm.^{2}						
							Uninoculated			Inoculated with *Azotobacter*			
							0	+P	+Ca	0	+P	+Ca	+P+Ca
75	1	Complete minerals + 43 lb. N per acre as amm. sulph. + 98 lb. N. per acre as rape cake	fallow side	5·2–5·6	0·5	0	0	0	(P−Ca) 0	0	0	+	+++
76	2		clover side	5·2–5·6	0–5	0	0	0	(P−Ca) 0	0	0	0−+	+++
77	3	Complete minerals (only to swedes)	fallow side	7·0–8·0	6–10	(*a*) 509 (*b*) —	0	35	0	—	—	—	—
78	4		clover side	7·0–8·0	6–10	(*a*) 390 (*b*) 439	0	15–20	0	—	—	—	—
79	5	Control	fallow side	8·0	6–10	(*a*) 340 (*b*) 300	0	20	0	—	—	—	—
80	6		clover side	7·8–8·0	0–5	(*a*) 83 (*b*) 66	0	*ca.* 5	0	—	—	—	—

and 5 which have a fallow included in their rotation, than from Plots 4 and 6 in which this fallow is replaced by clover.

As a contrast to the heavy Rothamsted soil, about ten samples of the light sandy soil from the Woburn plots were examined. *Azotobacter* was not found to be present in these soils, probably on account of their acidity. The kneaded-plate test was also applied to a sample of acid loamy soil from Cheshire, which was known to lack phosphate. This sample was remarkable in that no *Azotobacter* growth appeared on the kneaded plates even when the soil was previously inoculated with *Azotobacter* and when ample phosphate, $CaCO_3$ and mineral salts were supplied.

CONCLUSION.

The results of the phosphate test from seventy-nine soil samples from Rothamsted plots are summarised in Table X. The kneaded-plate test affords an indication of presence or absence of phosphate in those Rothamsted plots receiving little or no mineral nitrogen, but where soils have received 86 lb. or more of mineral nitrogen per acre, the size or viability of the *Azotobacter* population has usually been so depleted that they do not show up on the kneaded plates even when sufficient phosphate and lime are present. In sixteen of these samples the test was modified by adding *Azotobacter* from culture to the kneaded plates and, when this was done, the test gave a correct indication of phosphate supply or deficiency.

Table X. *Summary of results with the kneaded-plate test for phosphate deficiency.*

	P_2O_5 deficiency shown	No deficiency shown	Total tests	P_2O_5 deficiency shown	No deficiency shown	Total tests	P_2O_5 deficiency shown	No deficiency shown	Total tests	P_2O_5 deficiency shown	No deficiency shown	Total tests	Total samples tested
Water-soluble P_2O_5 mg. per kg.	0–5			6–10			11–15			>15			
Kneaded plates not inoculated	15	1	16	12	1	13	2	17	19	0	31	31	79
Inoculated with *Azotobacter*	6	0	6	2	0	2	0	2	2	0	6	6	16

SUMMARY AND ABSTRACT.

1. The kneaded plate (*plaque moulée*) method of detecting deficiency in lime and available phosphate was applied to seventy-nine soil samples taken from the classical Rothamsted arable plots, and the *Azotobacter* population from some of these samples was estimated by counts on silica jelly.

2. The silica jelly counts showed that *Azotobacter* cells were very much reduced in number, or even absent in soil receiving 86 lb. per acre or more of mineral nitrogen. It is suggested that this is due to competition with other organisms whose growth is stimulated by added nitrogen compounds.

3. The kneaded-plate test correctly indicated whether phosphate had been applied in soils receiving little or no nitrogen manures.

4. In those soils receiving 86 lb. or more of mineral nitrogen, the kneaded-plate test usually showed little or no *Azotobacter* growth even in the presence of phosphate and calcium carbonate. This failure was probably due to the paucity of *Azotobacter* cells originally present in such soil samples. In some cases the test was modified by inoculating the sample with a culture of *Azotobacter* and it then gave correct indications as to phosphate content.

5. In general, *Azotobacter* when present was found to develop on kneaded plates, if the soil contained at least 10 mg. of water soluble P_2O_5 per kilogram of soil, but below this limit little growth occurred.

6. The requirements of *Azotobacter* and of crop plants are similar as regards the need for soluble phosphate, but mineral nitrogen, although necessary to most crops, is detrimental to *Azotobacter* growth in field soil while soil acidity is more harmful to *Azotobacter* than to most crops. Consequently there was no correlation between crop yield and *Azotobacter* activity.

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APPENDIX.

APPLICATION OF WINOGRADSKY'S METHOD TO THE NITRIFICATION TEST.

The silica-plate method for estimating relative numbers of ammonia-oxidising organisms recently developed by Winogradsky (11) was applied to some of the soil samples collected in September 1931.

Method.

Petri dishes 10 cm. diameter, containing silica jelly, were prepared as described above, the gel impregnated with mineral salts and ammonium sulphate, and its surface covered with finely powdered $CaCO_3$. 10–20 mg. of finely sifted soil was distributed over the surface of each plate which was incubated at 27° C. After about 2 weeks,

Table XI. *Nitrification in Rothamsted Fields, September,* 1931.

Soil sample no.	Plot no.	Treatment	Number of ammonia oxidisers: colonies in 1 gm. soil	Yields in 1930. Grain, bushels per acre
Broadbalk:				
7	3	0	(*a*) 157 (*b*) 347	12
8	5	Minerals	(*a*) 500 (*b*) 312	14
9	8	Minerals + amm. sulph.	(*a*) 1922 (*b*) 1976	35
6	2	Dung every year	(*a*) 2243 (*b*) —	34
11	19	Rape cake + minerals	(*a*) 1428 (*b*) 1243	22
Hoos Field:				
37	1-O	0	(*a*) 245 (*b*) 457	14
38	4-O	Minerals	(*a*) 217 (*b*) 490	20
39	4-A	Minerals + amm. sulph.	(*a*) 353 (*b*) 150	41
40	4-AA	Minerals + nitrate	(*a*) 1333 (*b*) 640	39
41	4-C	Minerals + rape cake	(*a*) 3687 (*b*) —	39
42	7-1	Dung in 1852–71	(*a*) 664 (*b*) 168	24
43	7-2	Dung every year	(*a*) 3198 (*b*) —	46

colonies of ammonia oxidisers became apparent owing to their formation of clear haloes due to solution of the calcium carbonate.

In this work counts were made on duplicate plates which sometimes did not agree very well. Wherever examined, the ammonia-oxidising colonies were found to consist of cells resembling *Nitrosomonas*.

Results (Table XI).

Among the Broadbalk samples tested the highest counts of nitrifying organisms were obtained from Plot 2 receiving dung and from Plot 8 receiving sulphate of ammonia, though the figures from the rape cake Plot 19 were also high. On Hoos Field, dung and rape cake plots gave the highest counts, but here the low figure from the sulphate of ammonia plot 4-A, was probably due to soil acidity. On the nitrate plot two parallel plates disagreed but both gave higher figures than were obtained from Plot 4-A.

The plots with no nitrogen dressings both from Broadbalk and Hoos Field gave low counts of nitrifiers. In a neutral soil the population of nitrifying organisms thus seems to be dependent upon the nitrogen supply in the soil rather than upon its content of minerals. Thus, one would expect a better correlation with crop yield than was obtained with the *Azotobacter* tests. Table XI shows that there is indeed some relation between yield of crop and numbers of nitrifying bacteria, particularly in the case of Broadbalk.

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THE EFFECT OF *COLPIDIUM* ON AMMONIA PRODUCTION BY SOIL BACTERIA

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(With 8 Text-figures.)

INTRODUCTION.

THE problem of the effect of protozoa on bacterial activities is by no means a simple one. One aspect of this effect, namely the feeding or "phagocytic" action of protozoa, has been studied for a long time. It is well known that many species of protozoa derive most, if not all, of their nutriment from bacteria, and will reduce the numbers of bacteria in their surroundings by feeding upon them. It is when the chemical activities of the bacteria come to be considered that complications arise.

It might be supposed at first sight that, since protozoa will reduce the numbers of bacteria in a culture, the chemical activities of that culture will necessarily be reduced in their presence.

That this is not so in every case was shown by Cutler and Bal in the case of nitrogen fixation by *Azotobacter*, where an increased amount of nitrogen was found to be fixed in presence of protozoa (1).

The idea that reduction of bacterial numbers implies a decrease in the amount of a given product formed by the bacteria proceeds from the supposition, widely held, that the metabolic activity of a culture is simply proportional to the numbers of bacteria in that culture, that is that 2000 million bacteria will produce ten times as much of any given product as 200 million bacteria under the same conditions. This is tacitly assumed to be the case in much work, especially on soil bacteria, where the amount of some metabolic product is used as a measure of bacterial growth. In all such work the amount of a given product produced per individual organism is held to be constant, and independent of the numbers of bacteria present.

In a previous paper on ammonia production by a soil bacterium (6), a case was described where this is not so, the amount of ammonia produced per individual organism being found to be inversely proportional to the numbers of bacteria present. It followed from this that the actual amount of ammonia produced increased with the bacterial numbers up to a certain point, but numbers of bacteria above this "optimum" level produced actually less ammonia. The possible bearing of this relation between bacterial numbers and activity on the effects of the presence of protozoa in a bacterial culture was indicated by some preliminary experiments with a soil amoeba (*Hartmanella hyalina*). In these experiments the amoebae were found to reduce the numbers of bacteria in sand cultures (compared with control cultures in which no amoebae were present), in the majority of cases, while the ammonia production was found to be greater in the cultures containing amoebae. As a partial explanation of these results, it was suggested that the amoebae reduced the bacterial numbers from a value above the "optimum" level to one nearer to it, thus automatically increasing the amount of ammonia produced.

To obtain further data on this point the experiments described in this paper were carried out, in which liquid media were used, and the amounts of ammonia (and in some cases of carbon dioxide) produced by bacteria in the presence and absence of protozoa were noted. The species of protozoon used in these experiments was a ciliate, a species of *Colpidium*; the particular strain employed resembled very closely the strain of *C. colpoda* used by Cutler and Crump (2), and obtained by them from Peters. The dimensions of an average organism ($50\mu \times 25\mu$) agreed in both cases, but the undulating membrane round the mouth, which was easily seen in the Peters strain, is hardly visible in the one here described.

Colpidium was chosen for this work firstly because it was relatively easy to obtain and grow in culture, and secondly because it (or a related form) had previously been studied in this department (1, 2), and many of its properties were therefore well known.

In every experiment a culture containing *Colpidium* in company with a known bacterial flora was compared against a culture of the same bacteria alone, under identical conditions; the results obtained from the two series of cultures are, therefore, directly comparable.

In the choice of a medium two factors had to be considered, the suitability of the medium for the growth of *Colpidium*, and the presence of some nitrogenous compound to act as a source of ammonia.

The stock cultures of *Colpidium* were grown on soil extract, so the first series of experiments described here were carried out in a solution of peptone in soil extract. This medium, while it was suitable for the growth of *Colpidium*, was not considered satisfactory, as its composition was unknown and could not be exactly repeated at each making.

As a result of a series of trials with different simple nitrogenous substances, a medium containing alanine in a mineral salt solution was found to give satisfactory growth of *Colpidium*. This medium was of known composition and could be exactly repeated, and it was used in the second series of experiments.

It should be noticed that the bacterial numbers given throughout this work represent total counts, made with a counting chamber. It was felt that this method was to be preferred to a plate count, as there is no evidence that bacteria which are no longer capable of developing on the plate have ceased to show any metabolic activity.

METHODS. (*GENERAL.*)

In each experiment a pair of flasks, containing a known volume of liquid medium, was used. They were inoculated from parent cultures grown on soil extract, one flask of each pair with Colpidia and their accompanying bacteria, and the other with the same bacteria alone. The flasks were kept at room temperature, and the bacteria and protozoa in them counted daily. The ammonia in each culture was determined at intervals, and in some experiments a stream of air was passed through the cultures, and the carbon dioxide given off recorded.

The stock of *Colpidium* was obtained in April 1930, and was maintained on soil extract, made from farmyard manured soil. The early subcultures from the original stock culture were enriched with the bacterium YB (this enrichment has been repeated at every sub-culturing), and later attempts were made to obtain the Colpidia in pure culture with YB, by washing and inoculating the washed organisms into suspensions of YB in soil extract. These attempts, however, were not wholly successful; it was found impossible to eliminate one strain of bacteria which accompanied the Colpidia (referred to as strain CO). The *Colpidium* cultures used in this series of experiments contained, therefore, two strains of bacteria, YB and CO, the characteristics of which were as follows:

YB rods, $1{\cdot}6\text{–}2{\cdot}0\mu \times 0{\cdot}8\mu$, occasionally in pairs, non-motile, do not form spores, gram positive (weak); agar streak—growth abundant, smooth, yellow, opaque, medium unchanged; colonies on Thornton's medium—round umbonate, white or pale yellow, smooth shining, edge entire; liquefies gelatine slowly, produces acid on dextrose only (out of all sugars tested), no diastatic action on starch, reduces nitrates to nitrites; it will produce nitrite from ammonium phosphate in presence of dextrose or glycerine (there is evidence that this property has been only recently acquired); optimum temperature about 25° C. Total group number, according to the classification of the Society of American Bacteriologists—211·2442533.

CO rods, 2–4 $\mu \times 1 \mu$, occurring singly, motile, with peritrichous flagella, do not form spores, gram negative; agar streak—growth good, smooth, opalescent, medium unchanged; colonies on Thornton's medium—round flat, bluish translucent, edges undulate; liquefies gelatine quickly, produces acid on dextrose, saccharose, and glycerine, has no diastatic action on starch, reduces nitrates to nitrites; optimum temperature about 25° C. Total group number—211·2422032.

As a control, a mixed culture of the strains YB and CO was made from a soil extract culture in which the Colpidia had died. It was enriched with YB at each sub-culturing, in the same way as the *Colpidium* cultures, and a sub-culture of the same age as the corresponding *Colpidium* culture was used for each inoculation.

The method of inoculation employed was the same in every case; the numbers of bacteria in both parent cultures were estimated by a direct count. Volumes of each parent culture containing approximately the same bacterial numbers were then removed with a sterile pipette and added to the experimental flasks; in most cases 1 c.c. of the control culture, and 3 or 4 c.c. of the *Colpidium* culture, were taken. In the case of the control culture sterile soil extract was afterwards added to bring the final volume of liquid to the same level in both flasks.

Methods of counting. The counts in all cases were by the direct method. The bacteria in duplicate loopfuls were counted with a Thoma haemocytometer, 1 organism per small square representing a bacterial content of 20 million per c.c.: while the counting chamber used for estimating the numbers of Colpidia was of the Cropper type, 0·1 mm. in depth, with a ruled area of 25 sq. mm. divided into 625 small squares; owing to the small numbers of Colpidia it was usually necessary to make five counts on each occasion.

PART I. PEPTONE EXPERIMENTS.

Methods.

The medium used was a 0·5 per cent. solution of peptone (Bacteriological, B.D.H.), in soil extract. The soil extract was made by adding 2 litres of water to 1 kg. of soil, boiling for 1½ hours and filtering. In most cases the required volume of freshly made soil extract was placed in a conical flask and sterilised once in the autoclave, the peptone added, and the whole then steamed for 1 hour. In two cases (experiments 6 and

7), the soil extract used had been sterilised three times in 10 c.c. portions, before the peptone was added.

Eight experiments in all were performed, in the first three 60 c.c. of medium for each culture were used in a 250 c.c. conical flask, in the fourth and fifth 30 c.c. of medium in 100 c.c. flasks, and in the last three 50 c.c. in 250 c.c. flasks. In all cases the flasks were plugged with cotton wool and kept on the bench at room temperature. The full period of these experiments was a fortnight, but experiments 4, 5 and 8 were only continued for a week. Counts of bacteria and protozoa were made daily by the method described above.

The ammonia present in the cultures was determined at weekly intervals, on duplicate samples of 1 c.c. by the method of Woolf (9); the strength of the acid used for the titration was about 0·005 *N*, 1 c.c. being equivalent to 0·0711 mgm. of nitrogen as ammonia.

In one case (experiment 4) an additional ammonia estimation was made on the third day after inoculation.

Results.

As an illustration of the kind of results obtained, the course of two experiments (1 and 7) is shown in diagram form. In experiment 1 (Fig. 1) the Colpidia grew very well, reaching a maximum number of 5200 per c.c. and being still alive in appreciable numbers at the end of the second week

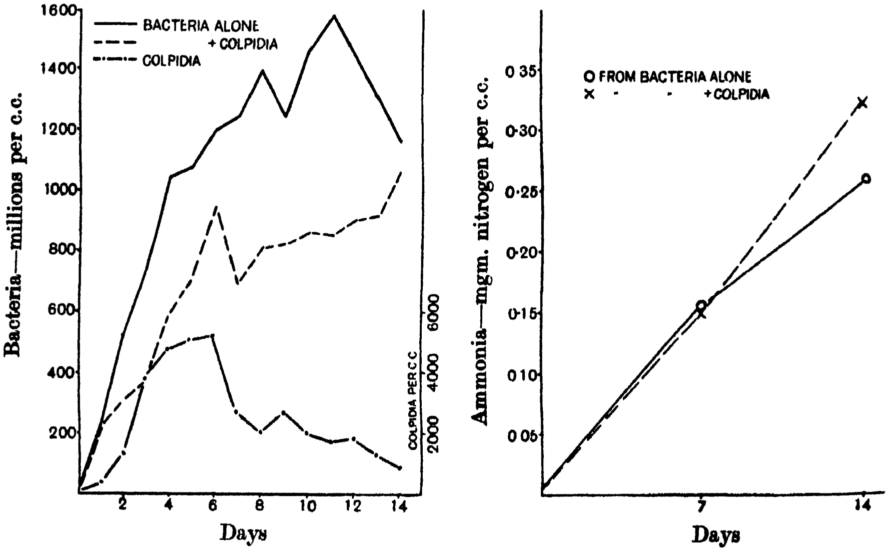

Fig. 1. Numbers of organisms and ammonia produced in experiment 1.

of the experiment. In consequence of this good growth of Colpidia, it will be seen that the numbers of bacteria were much reduced in the *Colpidium* culture; during practically the whole course of the experiment they remained below 1000 million per c.c., whereas in the control culture the numbers rose to 1580 million per c.c. and were above 1000 million from the fourth day onwards.

The ammonia present in the two cultures was practically the same in amount at the end of one week, and at the end of the second week more ammonia was present in the *Colpidium* culture than in the control.

Experiment 7, on the other hand, is an example of an experiment where the Colpidia did not grow well (Fig. 2). In this case they reached a

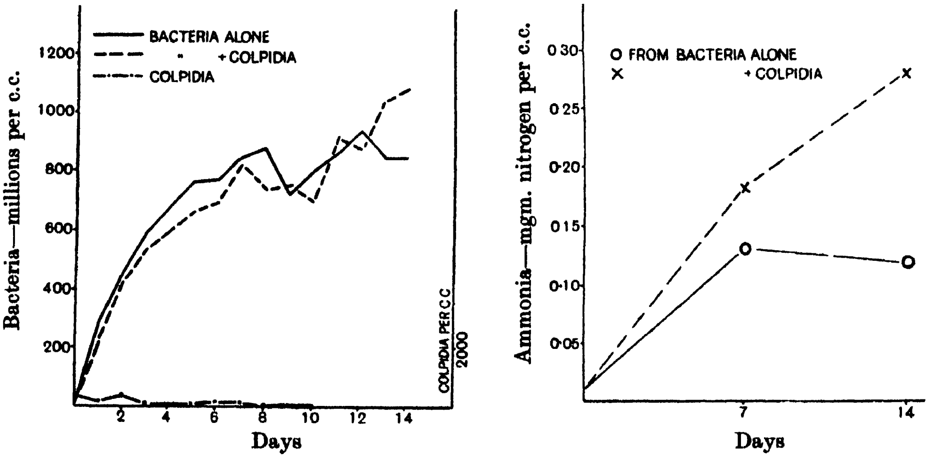

Fig. 2. Numbers of organisms and ammonia produced in experiment 7.

maximum of 320 per c.c. and at the end of a week were dying off, being reduced to 160 per c.c., and were all dead by the tenth day. The numbers of bacteria accordingly followed very nearly the same course in both cultures. There was, however, a greater amount of ammonia produced in the first week in the *Colpidium* culture than in the control; and this increase is seen to be continued in the second week, though, as some ammonia was lost from the control culture in the second week, the final estimation in this culture does not represent the full amount of ammonia formed.

The increase in ammonia in both sets of cultures during the first week of each experiment, and the average of the bacterial numbers over the first week, are shown in Table I (experiment 5 is omitted, for reasons to be explained).

Table I.

	Control cultures		*Colpidium* cultures	
Experiment	Average no. of bacteria (millions per c.c.)	Increase in ammonia (mgm. nitrogen per c.c.)	Average no. of bacteria (millions per c.c.)	Increase in ammonia (mgm. nitrogen per c.c.)
1	755	0·14	453	0·14
2	397	0·07	507	0·14
3	386	0·06	352	0·14
4	409	0·16	312	0·15
6	540	0·14	445	0·15
7	532	0·12	480	0·17
8	597	0·13	562	0·17
Average of all figures	517	0·12	444	0·15

It will be seen from Table I, that, though the Colpidia had reduced the numbers of bacteria as compared with those in the control cultures, yet the cultures containing Colpidia produced slightly more ammonia during the first week.

Since previous work on carbon dioxide production by Cutler and Crump (3), and on ammonia production by the writer (6), has shown that the efficiency of the individual organisms is a function of the numbers of bacteria present, it was decided to examine the relation between the numbers of bacteria in any culture and the ammonia produced in that culture. The method formerly used (6) was again employed; the efficiency of the individual organism was compared against the number of organisms, the amount of ammoniacal nitrogen produced in 24 hours per 1000 million bacteria being used as a measure of efficiency.

Table II.

	Control cultures			*Colpidium* cultures		
	n	P		n	P	
Experiment	Average no. of bacteria (millions per c.c.)	Ammonia per 24 hours (mgm. nitrogen per c.c.)	Q	Average no. of bacteria (millions per c.c.)	Ammonia per 24 hours (mgm. nitrogen per c.c.)	Q
1. 1st week	755	0·0202	0·0268	453	0·0197	0·0434
2nd ,,	1353	0·0148	0·0110	864	0·0242	0·0280
2. 1st ,,	397	0·0093	0·0235	507	0·0193	0·0397
2nd ,,	905	0·0207	0·0228	931	0·0205	0·0220
3. 1st ,,	386	0·0083	0·0216	352	0·0195	0·0554
2nd ,,	868	0·0171*	0·0197*	733	0·0163	0·0222
4. 1st ,,	409	0·0229	0·0560	161†	0·0220	0·1367
				467‡	0·0212	0·0454
5. 1st ,,	498	0·0210*	0·0421*	454	0·0276	0·0608
6. 1st ,,	540	0·0198	0·0367	445	0·0214	0·0481
7. 1st ,,	532	0·0170	0·0320	480	0·0245	0·0511
8. 1st ,,	597	0·0190	0·0318	562	0·0240	0·0428

* Values omitted from calculation. † First 3 days. ‡ 4th–7th days.

In Table II, the values of average bacterial numbers (n), ammonia produced per 24 hours (P), and efficiency (Q), are given for both sets of cultures.

From an inspection of Table II it will be seen, firstly, that the values of Q are higher, as a rule, for the *Colpidium* cultures, with an average value for Q of 0·0495, as against an average value of 0·0280 from the control cultures.

The second point to be noticed is that, on the whole, higher values of Q correspond to lower bacterial numbers in both sets of cultures. From this it appears that there probably is an inverse mathematical relation between Q and n (average bacterial numbers), and as the simplest case it may be presumed that this relation is a linear one. To test whether this relation does in fact exist, and is in fact linear, the regression of Q upon n was calculated.

On taking the figures for both sets of cultures together (except those marked * in Table II), the following results were obtained:

Mean value of $Q = 0{\cdot}0408$, if $n = 585$.
Regression coefficient of $(Q \times 10^4)$ on $n = -\,0{\cdot}6488$.
Estimated standard error of regression coefficient $= 0{\cdot}1711$.

From this value for the standard error, by applying Fisher's t test (4) the regression coefficient is found to be significantly different from zero, thus showing that the inverse relation presumed to exist between Q and n is a real one, and is linear. This relation can be expressed by the equation

$$Q \times 10^4 = 788 - 0{\cdot}649\, n.$$

On account of the small number of cases (only 21 for both series taken together) it was not possible, by investigating each series separately, to find whether the relation between Q and n in this case was altered by the presence of Colpidia.

It will have been noticed that two sets of figures in the control series (marked *) have been omitted from the calculation. This was on account of the detection of appreciable amounts of nitrite in these cultures; the causes of its formation are obscure, but as it was probably formed by the oxidation of ammonia, it is clear that the ammonia present in these cultures did not represent the total amount produced.

Numbers of Colpidia. It will be clear, from what has been said above, that the Colpidia did not grow equally well in all cases. While some of these differences in growth may be attributed to differences in the medium, their principal cause is undoubtedly the size and age of the inoculum, as is shown in Table III. In this table the cultures are arranged in order of the maximum numbers of Colpidia that were observed to be present in them.

Table III.

Experiment	Maximum no. per c.c. of Colpidia observed	Total no. of Colpidia in inoculum	Age of parent culture (days)	Life of Colpidia in experiment (days)
1	5200	22,000	4	14 +
8	1760	18,000	4	7 +
3	960	11,000	4	14 +
6	960	21,000	7	14 +
7	320	18,000	7	10
2	160	6,000	4	10
4	80	2,500	5	7
5	25	480	5	6

(Where a + sign is added in the last column, it means that the Colpidia were still alive on this day, which was the last on which observations were made.)

It will be seen that the maximum numbers attained follow the same order as the total number in the inoculum, except in the case of cultures 6 and 7, where the parent culture was older, and the maximum numbers attained were accordingly not so great as those from a younger parent culture of the same density. This dependence of the numbers of Colpidia attained on the size and age of the inoculum was recorded by Cutler and Crump (2).

Summary of results.

The results obtained from this first series of experiments may be summarised as follows:

1. In the presence of Colpidia the bacterial numbers are depressed, but a slightly greater amount of ammonia is produced.
2. Taking both sets of cultures together, an inverse linear relation is found to hold between average bacterial numbers and the amount of ammonia produced per organism.
3. The numbers of Colpidia in the experimental cultures were chiefly determined by the size of the inoculum and the age of the parent culture.

PART II. ALANINE EXPERIMENTS.

Methods.

The medium used was a 0·2 per cent. solution of alanine (α amino-propionic acid) in a mineral salt solution of the following composition: NaCl 0·06 per cent., KCl 0·001 per cent., $CaCl_2$ 0·002 per cent., $MgSO_4$ 0·001 per cent., KH_2PO_4 0·12 per cent., made up in ammonia-free distilled water. 50 c.c. portions of this medium were placed in 250 c.c. conical flasks, and steamed for 1 hour; the *p*H of the medium was then adjusted to 7·2 by the addition of $N/10$ sodium hydroxide, and it was steamed again for 1 hour.

Ten experiments in all were performed; the full period of an experiment was 2 weeks, but several experiments were not continued beyond the first week. The flasks were kept at room temperature, and in the first two experiments were plugged with cotton wool, but not otherwise aerated. In all the other experiments a slow stream of carbon dioxide-free air, filtered through cotton wool, was drawn through the liquid by an aspirator, at a rate of 5 to 7 litres of air per 24 hours. The carbon dioxide in the outgoing air was determined by the Pettenkofer method; a baryta solution of about 0·25 per cent. strength was used, and was titrated with approximately $N/10$ hydrochloric acid of known strength. Titrations were made at least once every 24 hours, and more often where necessary.

The amount of ammonia present in each culture was determined, by Woolf's method, on the third or fourth day of each experiment, at the end of a week, and at the end of 2 weeks when required. To detect any ammonia given off from the cultures, the outgoing air from each flask was passed through a trap containing 3 per cent. boric acid and 0·002 per cent. brom-cresol green.

Results.

In the first two experiments, in which the flasks were plugged with cotton wool, considerable amounts of nitrite were found in the control cultures, and the results of these experiments were accordingly rejected.

The results considered here are, then, those of the last eight experiments, of which three (experiments 3, 5 and 6) were continued for a fortnight, the others for a week only.

Growth of organisms. The course of events in a single experiment is illustrated in the following diagram of experiment 6 (Fig. 3). This experiment shows a double maximum in bacterial numbers in the control cultures, which was not observed in any other case, but is otherwise typical.

It will be seen that the Colpidia reach a maximum number of a little over 2000 per c.c. and survive till the eighth day. During the period when the Colpidia were active—the first 6 days—the numbers of bacteria were less in the *Colpidium* culture than in the control. In both cultures the numbers of bacteria rose till the tenth day, and fell off markedly after this point.

These two phenomena, the reduction of bacterial numbers by Colpidia in the first week, and the falling off in numbers during the second week, occurred in every case, and were borne out by the appearance of the cultures themselves.

The control cultures became progressively more turbid during the first few days, and a yellow colour developed in the medium from the fifth or sixth day onwards. In the *Colpidium* cultures, although an increasing turbidity was visible, it was not so great as that in the control cultures, and from the third to the fifth or sixth day the difference in turbidity between the two cultures was well marked; the yellow colour too began to develop a day or two later in the *Colpidium* cultures than in the controls. Towards the middle of the second week all the cultures began to clear, and the typical appearance of an old culture kept beyond the period of the experiment was of a clear, deep yellow, supernatant fluid, with a much reduced flocculent mass of bacteria on the bottom of the flask.

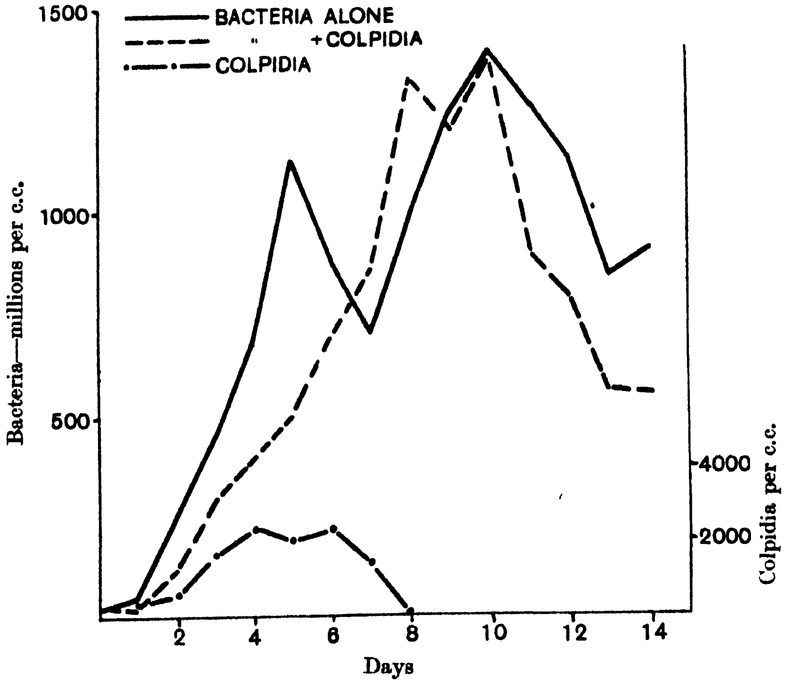

Fig. 3. Numbers of organisms in experiment 6.

The normal period during which Colpidia survive in the cultures seems to be 7 or 8 days, though in the last three experiments there were still over 1000 Colpidia per c.c. present at the end of a week, when the experiments were stopped. The maximum number of Colpidia recorded in individual cultures ranged from 2240 to 640 per c.c. with an average of 1400 per c.c.

Though the reduction in bacterial numbers by the Colpidia was very well marked, in most cases amounting to 40 to 60 per cent. of the control numbers during the first few days, yet there did not seem to be any simple relation between the amount of this reduction and the numbers of Colpidia present. This was probably due to the fact that the size of the Colpidia decreased with increasing age and increased crowding in the cultures.

Though the Colpidia reduced the total numbers of bacteria, they did not seem to affect the proportion of the two kinds of bacteria present. In the direct counts, the proportion of YB to CO bacteria in both series of cultures seemed to be about 3 to 1, and this was borne out by the relative numbers obtained in the plate count carried out on experiment 10.

On the fifth, sixth and seventh days of this experiment, in the control plates the CO colonies were 21 per cent., 29 per cent. and 26 per cent. of the total colonies, and in the *Colpidium* plates 28 per cent., 30 per cent. and 19 per cent. of the total.

The evolution of carbon dioxide. The amounts of carbon dioxide evolved from day to day in experiment 6 are shown in Fig. 4. In the control culture the carbon dioxide increased in amount till a maximum was reached on the fourth day of the experiment; the peak in the initial rise of bacterial numbers was observed on the fifth day. The amount of carbon dioxide then fell, to rise to a second peak on the eighth day, 2 days before the second peak in the bacterial numbers, observed on the tenth day. This occurrence of the maximum production of carbon dioxide a day before the maximum numbers of bacteria has been recorded by Cutler and Crump (3) and Telegdy-Kovats (8) in sand cultures, and was observed in eight cases in the series of experiments under discussion, five of the eight being control cultures.

In this connection it may be noted that an attempt was made to find whether any simple mathematical relation existed between the growth rate of the bacteria in a culture over a particular day, and the amount of carbon dioxide produced on that day. This attempt was not successful; in this series of cultures there appears to be no direct relation between the growth rate of the bacteria and the carbon dioxide produced by them.

The *Colpidium* culture evolved a greater amount of carbon dioxide than the control during the first 3 days, and the amount given off increased till the fourth day, when a slight fall was followed by a rise to a maximum value on the seventh day, corresponding to a peak in bacterial numbers on the eighth day. In both cultures the amount of carbon dioxide evolved fell off very much in the second week.

Efficiency relations. In studying the efficiency of the individual organism in the production of carbon dioxide, the amount of carbon dioxide produced per 24 hours per 1000 million bacteria (Q) was taken as a measure of efficiency. In Fig. 5 are shown the changes in this quantity, and in the average numbers of bacteria over every 24 hours, during the course of growth of a single culture, the control culture in experiment 5.

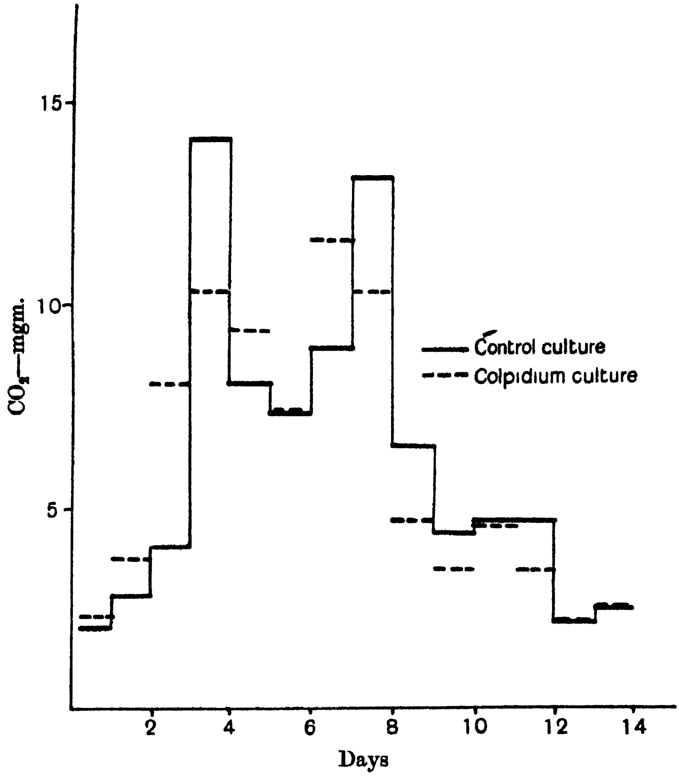

Fig. 4. Rate of evolution of CO_2—experiment 6.

The average bacterial numbers increased till the eighth day and then decreased; the efficiency fell while the bacterial numbers were increasing, and thereafter remained at a low and approximately constant level.

It is evident that the relation between efficiency and bacterial numbers undergoes a change after the bacterial numbers have reached their highest point and begun to decrease. It was therefore decided to study in detail only the period in which the bacterial numbers are still increasing, more particularly as the Colpidia were alive in the cultures during the earlier period only. The values of average bacterial numbers (n) carbon dioxide produced per c.c. per 24 hours (P) and efficiency (Q), up to the highest point of increase of the bacterial numbers, are given for six cultures (3–8), in Table IV. The quantity (Q) used as a measure of efficiency was calculated in each case from the equation

$$Q = \frac{P \times 1000}{n}.$$

The figures for the first 24 hours are omitted from the calculation in every case for the *Colpidium* cultures, and in two cases for the control cultures (omitted figures marked *), on account of the high possibility of error in the carbon dioxide estimation in these cases.

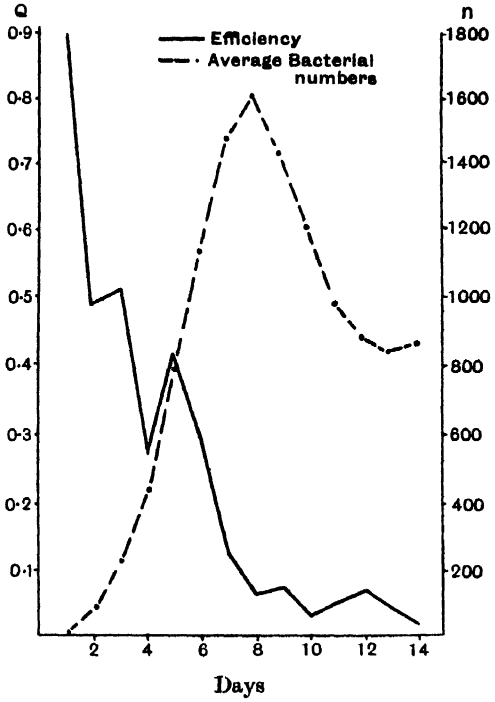

Fig. 5. Experiment 5—control culture.

The data were examined statistically with the following results:

Control cultures. Average value of $Q = 0{\cdot}3219$, if $n = 830$.
Regression coefficient of ($Q \times 10^3$) on $n = -\ 0{\cdot}3065$.
Estimated standard error of regression coefficient $= 0{\cdot}05515$.
Number of cases $= 41$.

From this value for the standard error, the regression coefficient is found by the t test to be significantly different from zero, and the relation

Table IV.

Carbon dioxide.

		Control cultures			Colpidia cultures		
Exp.	Days	*P* CO_2 per c.c. per 24 hours	*n* Average bacterial numbers (millions)	*Q*	*P* CO_2 per c.c. per 24 hours	*n* Average bacterial numbers (millions)	*Q*
3	0–1	0·0835	168·5	0·4955	0·0877	50	1·7540*
	1–2	0·1183	568	0·2083	0·1023	249	0·4108
	2–3	0·1981	1194	0·1659	0·2749	456	0·6029
	3–4	0·5850	1660	0·3524	0·2590	492	0·5264
	4–5	0·1279	1790	0·0715	0·2461	792	0·3107
	5–6	0·0831	1830	0·0454	0·1375	1282	0·1073
	6–7	0·0735	1835	0·0401	0·1693	1960	0·0864
	7–8				0·0799	2280	0·0350
4	0–1	0·0376	91	0·4132	0·0541	26	2·0810*
	1–2	0·1169	265	0·4411	0·0734	73·5	0·9986
	2–3	0·3421	680	0·5031	0·1087	268	0·4056
	3–4	0·4427	1430	0·3096	0·3012	624	0·4827
	4–5	0·0869	2060	0·0422	0·1773	940	0·1886
	5–6				0·2018	1330	0·1517
	6–7				0·1278	1750	0·0730
5	0–1	0·0162	18	0·9000	0·0362	15	2·4130*
	1–2	0·0439	90	0·4878	0·0596	28	2·1290
	2–3	0·1198	234	0·5120	0·0892	94	0·9489
	3–4	0·1183	436	0·2713	0·1822	229	0·7956
	4–5	0·3268	788	0·4147	0·3127	438	0·7139
	5–6	0·3302	1148	0·2876	0·2487	794	0·3132
	6–7	0·1881	1480	0·1271	0·1881	1235	0·1523
	7–8	0·0998	1610	0·0620	0·1238	1595	0·0776
6	0–1	0·0501	33	1·5180	0·0580	20	2·9010*
	1–2	0·0539	150	0·3593	0·0719	72	0·9986
	2–3	0·0770	354	0·2175	0·1540	208	0·7404
	3–4	0·2665	566	0·4708	0·1954	342	0·5713
	4–5	0·1616	900	0·1796	0·1864	448	0·4161
	5–6	0·1461	1000	0·1461	0·1461	600	0·2435
	6–7	0·1771	796	0·2225	0·2299	780	0·2947
	7–8	0·2888	862	0·3350	0·2269	1098	0·2066
	8–9	0·1443	1126	0·1282	0·1032	1266	0·0815
	9–10	0·0952	1312	0·0726	0·0748	1296	0·0577
	10–11	0·1021	1324	0·0771			
7	0–1	0·0443	23	1·9250*	0·0262	12	2·1800*
	1–2	0·0652	65	1·0030	0·0591	28	2·1090
	2–3	0·0632	212	0·2981	0·0632	63	1·0030
	3–4	0·1643	386	0·4258	0·1598	179	0·8926
	4–5		604			270	
	5–6	0·3420	792	0·4318	0·2611	358	0·7293
	6–7	0·2115	932	0·2270	0·2026	556	0·3644
	7–8	0·2337	1172	0·1994	0·2737	724	0·3781
8	0–1	0·0768	22	3·4900*	0·0536	16	3·3490*
	1–2	0·0939	72	1·3040	0·0819	38	2·1560
	2–3	0·1280	186	0·6878	0·0960	76	1·2640
	3–4	0·1280	346	0·3697	0·1559	130	1·2000
	4–5	0·1351	520	0·2597	0·1371	230	0·5959
	5–6	0·1475	704	0·2095	0·2057	384	0·5356
	6–7	0·2035	966	0·2106	0·2472	604	0·4093

* Values omitted from calculations.

between Q and n for the control cultures can be expressed by the equation

$$Q \times 10^4 = 5763 - 3{\cdot}065n.$$

Colpidium cultures. Average value of $Q = 0{\cdot}6039$, if $n = 644$.
Regression coefficient of $(Q \times 10^3)$ on $n = -0{\cdot}6819$.
Estimated standard error of regression coefficient $= 0{\cdot}1045$.
Number of cases $= 41$.

In this case also the regression coefficient is significantly different from zero, and the relation between Q and n for the *Colpidium* cultures can be expressed by the equation

$$Q \times 10^4 = 10430 - 6{\cdot}819n.$$

The relation between efficiency and bacterial numbers in the case of carbon dioxide production is therefore of the same kind as that formerly recorded for ammonia production, that is, that Q falls off regularly for increasing values of n.

Though the two sets of cultures show the same kind of relation between Q and n, yet there is a significant difference between the regression coefficients for the control and the *Colpidium* cultures, as is shown in Table V.

Table V.

	Regression coefficient	Estimated standard error	t	Degrees of freedom
Control cultures	-0·3065	0·0552	5·56	39
Colpidium cultures	-0·6819	0·1045	6·53	39
Difference	0·3754	0·1181	3·18	—

The value for t obtained for the difference shows it to be significant.

In Fig. 6 the calculated regression lines for both sets of cultures are shown, with the observed values represented by dots. In the case of the control cultures, the dots are evenly distributed about the line, which can be considered to represent adequately the relation between Q and n. With the *Colpidium* cultures, however, the points do not fit so well, particularly at very low or very high values of n, and although it was assumed, for purposes of calculating the regression, that the relation between Q and n was a linear one, it is probable that this relation would be more accurately expressed by a hyperbolic curve.

Effect of time. It will be noticed in Fig. 5 that the average bacterial numbers are increasing with time over the period studied, while the efficiency is decreasing. It may therefore be doubted whether the negative relation found to hold between Q and n may not be entirely due to the effect of time on these two quantities.

To test this point the observations for each set of cultures were grouped in days, and the variations from one day to another separated from the variations within days, when the following results were obtained (Table VI).

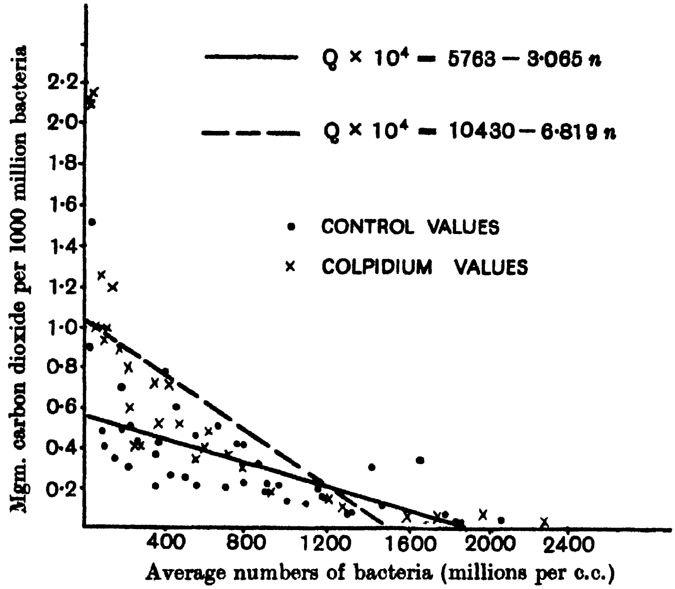

Fig. 6. Carbon dioxide—relation between bacterial numbers and efficiency.

Table VI.

	Number of days	Regression coefficient	Estimated standard error	t	Degrees of freedom
Control cultures	11				
Total		−0·3065	0·0552	5·56	39
Within days		−0·1864	0·0765	2·44	29
Colpidium cultures	9				
Total		−0·6819	0·1045	6·53	39
Within days		−0·4270	0·1566	2·73	31

By applying the t test (4) the regression coefficients due to variation within days are seen to be significant.

When the effect of time is eliminated there remains in both cases a significant negative relation between Q and n; that is to say, that though a culture 7 days old will have a lower efficiency than one 4 days old, yet of two 4-day-old cultures the one with the lower bacterial numbers will have the higher efficiency.

There are not sufficient data to show whether the regression independent of time is significantly different in the two sets of cultures.

Ammonia production. The results obtained for ammonia production from this series of cultures were very similar to those obtained on peptone. The eight control cultures produced an average of 0·19 mgm. of ammonia nitrogen per c.c. in one week, with an average bacterial content for all control cultures of 662 millions per c.c. In the *Colpidium* cultures the average ammonia nitrogen produced at the end of one week was 0·18 mgm. per c.c. from an average bacterial content of 398 millions per c.c. for all cultures.

In the *Colpidium* cultures, therefore, although the bacterial numbers were sensibly reduced, the amount of ammonia produced was nearly the same as from the control cultures.

The values of average bacterial numbers (n), ammonia nitrogen per c.c. produced per 24 hours (P) and efficiency (Q) for both series of cultures are given in Table VII.

The quantity Q used as a measure of efficiency was calculated as before. (Ammonia nitrogen per 24 hours per 1000 million bacteria.)

Table VII.

Ammonia nitrogen.

		Control cultures			*Colpidium* cultures		
Exp.	Days	n Average bacterial numbers (millions)	P Ammonia N (mgm. per c.c.)	Q	n Average bacterial numbers (millions)	P Ammonia N (mgm. per c.c.)	Q
3	1–7	1247	0·0343	0·0275	815	0·0314	0·0386
4	1–3	386	0·0571	0·1479	147	0·0255	0·1735
	3–7	1702	0·0135	0·0079	1162	0·0381	0·0328
5	1–3	126	0·0193	0·1532	54	0·0135	0·2500
	3–4	436	0·0324	0·0743	229	0·0298	0·1301
	4–7	1134	0·0445	0·0392	837	0·0545	0·0651
6	1–4	291	0·0230	0·0790	169	0·0199	0·1177
	4–7	848	0·0269	0·0317	614	0·0351	0·0572
7	1–3	118	0·0102	0·0864	37	0·0054	0·1459
	3–7	681	0·0382	0·0561	384	0·0335	0·0963
8	1–4	169	0·0178	0·1053	69	0·0117	0·1688
	4–7	743	0·0310	0·0418	417	0·0366	0·0877
9	1–7	500	0·0218	0·0435	171	0·0161	0·0939
10	1–7	387	0·0198	0·0512	186	0·0177	0·0953

A statistical analysis of the two sets of data gave the following results:

Control cultures. Average value of $Q = 0·0675$, if $n = 627$ million.

Regression coefficient of $(Q \times 10^4)$ on $n = -0·707$.

Estimated standard error of regression coefficient $= 0·1745$.

$t = 4·05$.

Number of cases = 14.

Colpidium cultures. Average value of $Q = 0{\cdot}1109$, if $n = 376$ millions.
Regression coefficient of $(Q \times 10^4)$ on $n = -1{\cdot}35$.
Estimated standard error of regression coefficient $= 0{\cdot}2976$.
$t = 4{\cdot}54$.
Number of cases = 14.

Difference in regression coefficients $= 0{\cdot}643$.
Estimated standard error of difference $= 0{\cdot}2411$.
$t = 2{\cdot}667$.

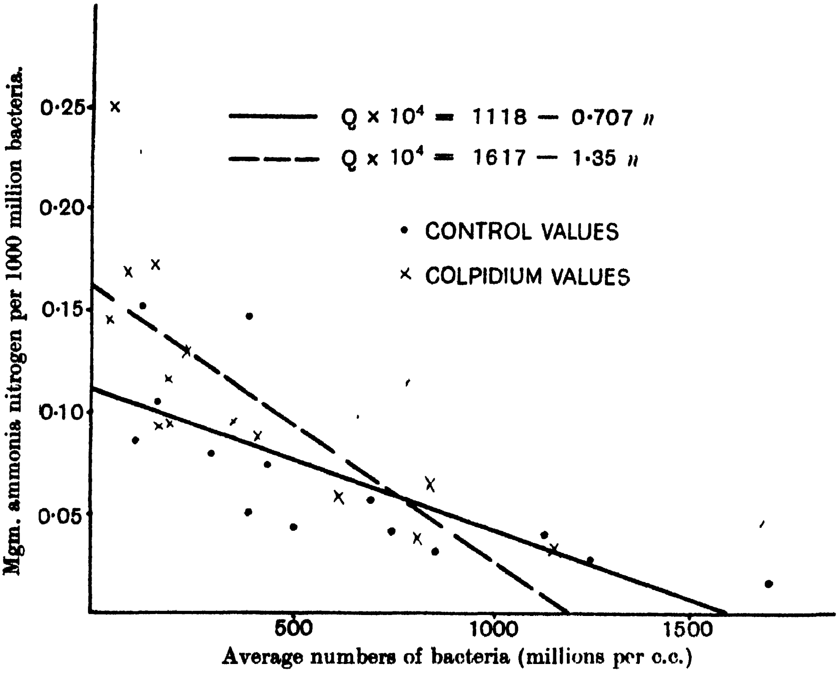

Fig. 7. Ammonia—relation between bacterial numbers and efficiency.

From the values of t in each case it is apparent that there exists a significant negative relation between Q and n for both the *Colpidium* and the control cultures, and that the two regressions are significantly different.

The relation between Q and n for the control cultures can be expressed by the equation

$$Q \times 10^4 = 1118 - 0{\cdot}707n,$$

and for the *Colpidium* cultures by the equation

$$Q \times 10^4 = 1617 - 1{\cdot}35n.$$

In Fig. 7 the actual observations for both sets of cultures are shown together with the calculated regression lines, which seem to give a fairly accurate picture of the true relation between Q and n.

There are not sufficient data to discover how much of the variations of Q and n are due to the effect of time, and to eliminate this effect.

Changes in medium during course of experiment. In two experiments (9 and 10), in which pH measurements were made daily by the capillator method, the cultures were found to become steadily more alkaline, the pH changing from 7·1 at the start to about 8·0 at the end of a week. The alkalinity corresponds roughly to the amount of ammonia present, the culture that contains more ammonia being more alkaline.

In Fig. 8 are shown the total carbon dioxide and total ammonia nitrogen produced up to any day in experiment 6. The scale on which each product is plotted has been adjusted so that two mols of carbon dioxide correspond to each mol of ammonia. It is evident that at the end of a fortnight the reactions taking place in the culture are practically completed; the bacteria have begun to die off before this, and the carbon dioxide produced per day has fallen to a very low value.

If all the carbon in the medium were converted into carbon dioxide the total carbon dioxide evolved would be about 148 mgm.; the amount of carbon dioxide actually evolved in a fortnight corresponds to two-thirds of this figure. In experiment 6, for instance, two-thirds of the total carbon as carbon dioxide would give 89·8 mgm. of carbon dioxide in all (allowing for carbon lost in sampling). The total carbon dioxide actually evolved in a fortnight was 85·6 mgm. from the control culture, and 84·2 mgm. from the *Colpidium* culture. At the completion of the reaction, or set of reactions, carried out by the bacteria, two-thirds of the total carbon is accounted for as carbon dioxide. As will be seen from Fig. 8, during the first week one mol of ammonia is produced for every two mols of carbon dioxide, as the points representing ammonia produced lie along the curve of carbon dioxide production. Now the alanine molecule contains three carbon atoms to one nitrogen atom—its structural formula being CH_3CHNH_2COOH, and the proportion of carbon dioxide to ammonia produced suggests that the main reaction taking place is one that involves the break up of the carbon chain, two carbon atoms being lost as carbon dioxide.

With regard to the ammonia, it should be emphasised that the ammonia recorded was all present in the medium; in no case was there any loss of ammonia from the cultures during the first week, and only very small losses were recorded by the acid traps in the second week. It is possible that the ammonia is present in the medium as ammonium bicarbonate, and that the third carbon atom from each alanine molecule is also accounted for in the formation of this compound. This supposition is rendered more probable by the increasing alkalinity of the medium, and also by the fact that the filtrate from an old culture, on titration to *p*H 6·0 and subsequent aeration in the cold, gave off an appreciable

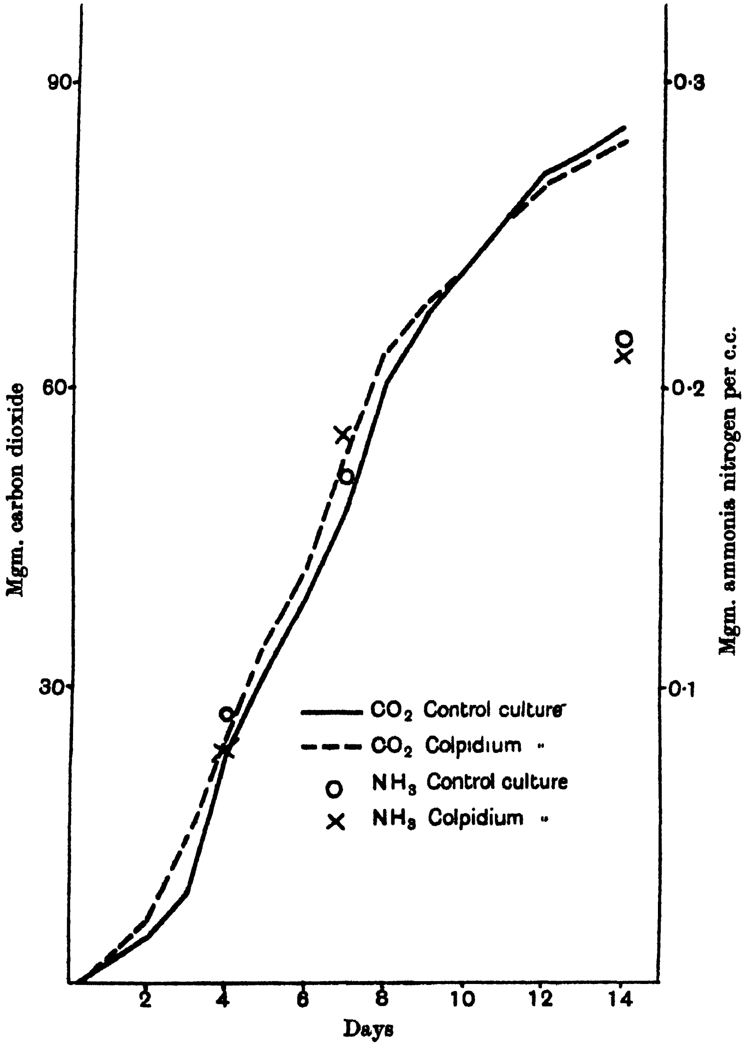

Fig. 8. Total carbon dioxide and ammonia in experiment 6.

amount of carbon dioxide; a solution of ammonium carbonate of corresponding strength behaved in the same way.

That there were other reactions taking place in the culture besides this complete breakdown with formation of ammonium bicarbonate, is shown by the fact that in the residue from an old culture there are present a volatile reducing substance, probably acetone, and small amounts of volatile acids, possibly acetic and formic acids, as salts.

Of the carbon not accounted for as carbon dioxide, only a very small proportion is used in the formation of bacterial protoplasm. If it is assumed that 20 per cent. of the total volume of the bacteria is solid matter, and that 50 per cent. of this is carbon, then at the greatest concentration of bacteria in these experiments, 2000 million per c.c., the weight of carbon in the substance of the bacteria is only 0·2 mgm., whereas there are 13·5 mgm. of carbon in the medium not accounted for as carbon dioxide.

It is of interest to note that the ratio $\dfrac{\text{nitrogen as ammonia}}{\text{carbon as carbon dioxide}}$ is higher at the end of a week in the control culture than in the *Colpidium* culture in every case but one; the mean value of this ratio is 0·685 for the control cultures, and 0·596 for the *Colpidium* cultures, and the difference, though small, is significant.

DISCUSSION.

On considering the foregoing results, there are two main conclusions which emerge. The first is that in any one series of experiments, the amount of ammonia, or of carbon dioxide, produced per individual organism (efficiency) is found to fall off regularly as the bacterial numbers increase. The regression of efficiency on bacterial numbers is represented very nearly by a straight line, except in the case of carbon dioxide production in *Colpidium* cultures, where the values of efficiency for very low bacterial numbers are higher than would be expected, and a curved regression line would fit the data better. This raising of efficiency for low bacterial numbers may be partly due to the respiration of the Colpidia, but it does not show any relation to the numbers of Colpidia present.

The inverse relation between efficiency and bacterial numbers agrees with the results recorded in a previous paper (6), although the present experiments were not performed with a pure culture of bacteria, and were of much shorter duration than in the former case.

The second conclusion is that, although the numbers of bacteria in the *Colpidium* cultures are markedly lower than in the controls, yet the two sets of cultures produce nearly equal amounts of ammonia or of carbon dioxide; that is, that the *Colpidium* cultures show a higher efficiency. Now it might be supposed that, since efficiency is inversely proportional to bacterial numbers, the higher efficiency observed in the

Colpidium cultures was due entirely to their lower bacterial numbers. If this were so, then one would expect the same regression line of efficiency on bacterial numbers to fit the data from both *Colpidium* and control cultures. The two sets of cultures, however, give regression lines which are significantly different; the effect of the presence of Colpidia on ammonia or carbon dioxide production is not entirely due to the reduction of bacterial numbers to a point nearer the optimum level for metabolic activity. There remain two explanations for this increased efficiency; the extra carbon dioxide or ammonia may not be produced by the bacteria, but by the Colpidia; or the presence of the Colpidia may exert a stimulating effect on bacterial activity, over and above that caused by the lower numbers of bacteria present. The first alternative involves the assumption that the Colpidia are partially saprophytic in their method of feeding; the quantities of carbon dioxide and ammonia produced are too large to be the products of respiration and excretion of a holozoic organism. The majority of work on the feeding habits of *Colpidium*, however, seems to show that it is undoubtedly holozoic; Cutler and Crump(2) found that the rate of growth of a *Colpidium* culture was dependent on the number of bacteria supplied to it, and Oehler(7), who made several attempts to grow it in bacteria-free media, though he obtained sterile specimens, never succeeded in getting growth and multiplication except with living bacteria as a source of food. Luck, Sheets and Thomas(5), however, report that they have cultivated *Colpidium* in bacteria-free media, but give no details. In the present series of cultures, the marked reduction in bacterial numbers produced by the Colpidia, and the presence of bacteria in their food vacuoles, suggest that the Colpidia are predominantly, if not entirely, holozoic.

There remains the possibility of a stimulating effect due to the presence of Colpidia. Such a stimulus might be due to the formation by the protozoa of an excretion product (*e.g.* urea), which was more easily utilised by the bacteria than the original substrate. The existence of such an excretion product, though quite possible, cannot be detected, as it would be decomposed by the bacteria as fast as it was formed. Apart from the possibility of such an excretion product, the Colpidia may exert a stimulating action by keeping the cultures in which they are present in a state of physiological youth over a longer period than the normal. A normal culture of bacteria shows a high level of metabolic activity during the early stages, in which the bacteria are actively dividing, but in the later stages, when the multiplication of bacteria slows down, and eventually stops, this activity is considerably lessened. If protozoa are present, however, they exert what may be described as a "pruning" action on the culture; by removing bacteria from the culture by feeding, they are compelling the remaining bacteria to divide more rapidly. A culture in which protozoa were present would therefore be in a state of active division over a longer period than the normal, and might therefore exhibit the high metabolic activity of a very young culture during this period. This hypothesis was put forward by Cutler and Bal (1) to explain the increase in nitrogen fixation by *Azotobacter* due to the presence of Colpidia. In the present case it seems to provide at any rate a partial explanation of the undoubted stimulating effect of Colpidia on ammonia production.

SUMMARY.

1. Two series of experiments were performed with a mixed culture of two species of soil bacteria with and without Colpidia, one series on a solution of peptone in soil extract, the other on a synthetic medium containing alanine.
2. An appreciable reduction in bacterial numbers was found on both media to occur in the *Colpidium* cultures as compared with the controls.
3. On the peptone medium the *Colpidium* cultures produced slightly more ammonia than the controls, in spite of their lower numbers of bacteria.
4. On the alanine medium the *Colpidium* cultures produced nearly the same amount of carbon dioxide and of ammonia as the controls.
5. In both series of experiments an inverse linear relation was found to exist between bacterial numbers and efficiency of ammonia production, and the same was found for carbon dioxide production in the second series.
6. The regression coefficients of efficiency on average bacterial numbers are significantly different in the *Colpidium* and control cultures fo the second series.
7. It follows that the stimulating effect of the presence of Colpidia is not due solely to the reduction of bacterial numbers to an optimum value, and it is suggested that in the cultures in which Colpidia are present, the bacteria are kept in a state of youth for a longer period.

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SOME FACTORS INFLUENCING THE DISTRIBUTION OF CERTAIN PROTOZOA IN BIOLOGICAL FILTERS

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I. INTRODUCTION.

DURING the past four years investigations have been carried out to discover whether the principle of biological purification on percolating filters could be applied to the waste effluent from the beet sugar factories. At an early stage in the research it was found that large numbers of Protozoa, as well as bacteria and other organisms, were inhabitants of such filters. At first the work was done on a large scale at a factory, but when it was transferred to the laboratory with specially designed filters (1) it was thought of interest to make frequent observations of the protozoan species, since opportunity would thus be afforded for studying the effects of changes in hydrogen-ion concentration and in chemical composition of the medium on the fauna. Knowledge of the ecology of Protozoa has mostly been derived from the study of inland waters and soil, where the conditions could not be varied at will, or from culture solutions under laboratory conditions. In the filters, on the other hand, which depend for their population on chance contaminations and therefore approximate to natural conditions, the medium can be changed at will and therefore a good opportunity is afforded for work of this type.

Records have been kept of the following types of Protozoa occurring in the filters, and as, in some cases, the species have not been identified only the generic names are included in the list.

Sarcodina

Genus	No. of species
Amoeba	3
Vahlkampfia	2
Naegleria	1
Hartmanella	1
Sappinia	1
Pelomyxa	1
Arcella	1
Hyalosphenia	1
Euglypha	1
Cochliopodium	1

Mastigophora

Genus	No. of species
Bodo	2
Heteromita	2
Cercomonas	1
Oicomonas	1
Trepomonas	1
Proleptomonas	1
Astasia	1
Entosiphon	1

Ciliata

Genus	No. of species
Colpoda	2
Colpidium	2
Glaucoma	2
Chilodon	2
Paramoecium	2
Lionotus	2
Vorticella	2
Pleuronema	1
Histrio	1
Spathidium	1
Oxytricha	1
Uroleptus	1
Epistylis	1
Podophrya	1
Cinetochilum	1

In this paper only the following species have been considered as regards the effect of the varying conditions: *Bodo saltans* Ehrbg., *Trepomonas agilis* Dujardin, *Arcella vulgaris* Ehrbg., *Colpidium colpoda* Stein, *Paramoecium putrinum* Clap. and Lach., *Pleuronema chrysalis* Ehrbg., *Cinetochilum margaritaceum* Ehrbg., *Lionotus fasciola* Ehrbg.

II. METHODS.

The object of the experimental filters was to convert a 0·2 per cent. solution of sucrose into carbon dioxide and water in the shortest possible space and time. Each filter consisted of six earthenware pipes each 12 in. long by 4 in. diameter; these were arranged in vertical series, each separated from the next by a space deep enough to allow of sampling the effluent from each section. The filters were filled with gravel (¼–⅜ in.=0·6–1 cm.), and the sections were seeded with some of the film obtained from larger experimental filters which had been working at the Colwick factory of the Anglo-Scottish Beet Sugar Corporation for two years, and which therefore contained a representative fauna and flora. The filters were fed with about 5 litres of solution in 24 hours from an aspirator fitted with a Mariotte's constant head device. The solution unless otherwise stated had the following composition:

Sucrose	0·2	per cent.
NaCl	0·03	„
K_2HPO_4	0·03	„
KH_2PO_4	0·02	„
$MgSO_4$	0·01	„
$(NH_4)_2SO_4$	0·03	„
$CaCO_3$	0·03	„

A record was kept of the changes in acidity in each section and of the purification, as evidenced by the test for dissolved oxygen taken up in 5 days, which is a measure of the amount of reducing material present in the solution (**3**).

The decomposition of carbohydrates and ammonia in their passage through the filters is roughly as follows (**1**, **2**): in the first section the greater part of the sugar is converted into various organic acids, among which lactic, pyruvic, acetic and formic may occur; in the second and third sections these acids undergo oxidation, and in the fourth, fifth and sixth sections practically no carbohydrates are present; the ammonia disappears after the first section and amino acids are built up in the second section and to a lesser extent in the third; in the fourth section ammonia appears again from the amino acids and this is subsequently converted into nitrites and nitrates. Crudely therefore the sections may be classified as follows:

1. Organic acids, *p*H under 7·0.
2. Amino acids, *p*H under 7·0.
3. Amino acids decreasing, *p*H about 7·0.
4. Amino acids decreasing, appearance of nitrites and nitrates, *p*H over 7·0.
5. Very little organic matter, appearance of nitrites and nitrates, *p*H over 7·0.
6. Very little organic matter, appearance of nitrites and nitrates, *p*H over 7·0.

III. RESULTS.

The five principal factors which may be involved in determining the protozoan distribution are: hydrogen-ion concentration, purification, food supply, direct chemical action, and lastly the presence of large numbers of particular bacteria, developing in the solution in response to the presence of certain chemical substances.

Purification. As judged by the five-day tests the percentage of oxidisable material decreased from the top of the filter (section 1) down to the bottom (section 6), and to discover what effect this had on the Protozoa observations were made on the various sections. The percentage of occurrences of the different species of Protozoa varied with the different levels of the filter as is seen from Table I. In this table the percentage occurrences were calculated on the total number of observations made, that is the total number of times the organism might have occurred, in each section. The figures quoted in the tables bear no reference to the relative abundance of the animals on any one day, since presence or absence alone were recorded. It is difficult in such material to estimate actual numbers with any degree of accuracy, but under exactly similar conditions the numbers fluctuate to a very marked extent.

Table I. *Distribution of the species of Protozoa through the filters.*

	Section 1		Section 2		Section 3		Section 4		Section 5		Section 6	
	No. of cases	% occurrence	No. of cases	% occurrence	No. of cases	% occurrence	No. of cases	% occurrence	No. of cases	% occurrence	No. of cases	% occurrence
Bodo	275	39·6	273	36·9	273	34·4	271	29·9	265	28·8	270	26·3
Trepomonas	244	36·9	250	40·0	247	28 7	245	21·2	249	17·6	245	14·3
Colpidium	274	64·6	274	74·4	274	59·1	274	58·0	274	39·0	274	27·0
Paramoecium	262	67·1	266	80·1	267	80·5	266	78·5	266	69 9	267	62·9
Pleuronema	271	56·1	270	61·5	270	69 6	270	54·1	270	45·2	270	41·1
*Arcella	162	11·7	162	22·8	162	40·7	162	67·6	162	71·2	134	72·4
*Cinetochilum	148	21·6	148	27·0	148	43·9	148	68·9	148	69·9	112	65·2

* These two species differ from the rest in that they were not present in all filters and the results for the periods when they were known not to occur have been omitted.

It will be noted that the protozoan species can be divided into three groups, firstly those like *Arcella* and *Cinetochilum*, which prefer the lower levels of the filter, secondly those like *Trepomonas*, *Colpidium* and *Bodo*, which are found mostly in the upper layers; and thirdly those which are comparatively indifferent, like *Paramoecium* and possibly *Pleuronema*.

This distribution is undoubtedly influenced by two main factors: the purity of the medium, and the food supply. *Cinetochilum* which feeds on organic débris, small organisms and bacteria (4) could undoubtedly obtain a more adequate food supply higher in the filter; it is therefore clear that it requires an environment relatively free from soluble organic compounds and so is limited in its range by the conditions of the effluent. On the other hand it is very problematical whether *Colpidium*, which feeds almost exclusively on bacteria (4, 5), could obtain a living in the bottom sections, where the bacterial flora is greatly reduced, quite apart from any considerations of the purity of the medium, and so in this case the food supply is possibly the limiting factor. This was borne out by observation, for the animals in the lower sections were small and obviously starved.

Further information on these points was obtained when the filters were receiving only tap water. During this period *Colpidium* and *Bodo*, which commonly occur at the top, were reduced in numbers, while the percentage of occurrences of *Cinetochilum* changed from 10·7 in the sucrose solution to 65·5 in the tap water. The percentage occurrence of *Paramoecium* was comparatively unchanged (Table II).

Table II. *Percentage occurrences of species during* 14 *days with sucrose solution and* 14 *days with tap water, all sections added together.*

Species	Total No. of observations	0·2 % sucrose	Tap water
Paramoecium putrinum	84	77·4	82·1
Cinetochilum margaritaceum	84	10·7	65·5
Colpidium colpoda	84	77·4	19·1
Bodo saltans	84	78·6	17·8

Effect of p*H values.* The *p*H values in the various sections of the filters ranged from 4·3 to 8·0; the acid conditions being found more often in the first two sections. Tables III–IX have therefore been prepared from separate sections, so as to eliminate other variables, such as purification, as far as possible. The majority of the Protozoa found are able to live throughout the range of *p*H values, provided they are not adversely affected by other conditions, such as impurity, though in many cases the low or high *p*H values have a limiting effect. As the behaviour of the species is different, consideration of each one individually is necessary.

Trepomonas. As is seen in Table I this species is not an ubiquitous inhabitant of the filters; but when it does occur its distribution is largely affected by the purity of the solution. The *p*H values *per se* have no marked effect (Table III).

Colpidium. Here again the distribution is little affected by *p*H values though there is some evidence that neutrality or slight alkalinity are the most suitable conditions (Table IV).

Paramoecium. This species is practically indifferent to section distribution and occurs through a wide range of *p*H values, though less frequently in the more acid groups (Table V).

Pleuronema. In the case of this animal the distribution through the sections is similar to that of *Paramoecium* except that there is a more definite falling off in the first and last sections. This is of interest in view of the fact that there is a narrower *p*H range in which *Pleuronema* occurs. As is seen from the table the optimum range lies between 6·5–7·6, and as the *p*H values outside this range tend to occur in the top and bottom sections the lower numbers of occurrences in these two situations are explained (Table VI).

Arcella. *Arcella* shows a very marked preference for the lower sections of the filter. In these sections (5 and 6) it occurs freely at all *p*H values; nevertheless in these two sections and still more in sections 1 and 2 there is definite evidence that it prefers an acid medium (Table VII).

Cinetochilum. *Cinetochilum* also shows a preference for the three lower sections; but in this case, although it can occur freely at *p*H values down to 6·1 greater degrees of acidity definitely act as limiting factors (Table VIII).

Bodo. This particular species shows a slight falling off in numbers at the bottom of the filter; but like *Trepomonas* it is not one of the commoner forms found. As regards its *p*H distribution it exhibits curious irregularities, for reference to the table demonstrates that maximum development occurs at an acid value of about 5·3 and again at an alkaline one of about 7·6. It is possible that this peculiarity was due to the presence of different physiological strains, but the evidence is insufficient to establish this with certainty (Table IX).

Effect of food supply. The effects of three changes in the composition of the solution to be filtered were studied, these changes being the omission of phosphate, the substitution of urea for ammonium sulphate, and of lactic acid for sucrose. The period during which phosphate was omitted was only a short one, 11 days: and though only tentative conclusions can be drawn from such limited data, it was thought worth while to present the results. Table X gives the percentage of occurrences of the different species during the period when the filter was receiving no phosphate compared with the previous 11 days when the population was receiving a normal diet. In general the omission of phosphate causes a reduction in numbers, but *Colpidium* and *Cinetochilum* are exceptions to this rule. The slight falling off of the other species is what might be expected owing to the lowering of the bacterial numbers and therefore the food supply; but this would only be a gradual effect as the filter for the first few days had reserves of phosphate. Why this effected *Colpidium* and *Cinetochilum* in the reverse manner is inexplicable.

The effect of urea (Table XI) was a depressing one on most species though again *Colpidium* was increased as was *Pleuronema*. These results are interesting, though difficult of explanation, since the expectation would be that urea would have no effect, for it normally breaks down to give salts of ammonia.

Another set of conditions connected with a change in the food supply arose when lactic acid was given to one filter instead of sucrose, the object being to study the effect of extreme acidity on the protozoan population. The average *p*H value of the effluent from the top section during the period when lactic acid was given was 4·0, but in all the other sections the effluents showed no increase in acidity above the normal values that were found when the filters were receiving sucrose. As is seen from Tables III–IX a *p*H value of less than 4·9 limits the occurrence of all the protozoan species under

Table III. Trepomonas agilis. *Effect of* p*H values.*

	Section 1		Section 2		Sections 5 and 6	
*p*H range	No. of cases	% occurrence	No. of cases	% occurrence	No. of cases	% occurrence
Under 4·9	27	0·0	8	25·0	—	—
4·9–5·2	5	40·0	5	60·0	—	—
5·3–5·6	17	41·1	17	58·7	16	33·3
5·7–6·0	36	41·7	39	46·1	2	50·0
6·1–6·4	23	52·3	36	47·2	21	14·2
6·5–6·8	70	62·0	115	40·0	59	8·4
6·9–7·2	38	55·1	66	39·4	146	18·5
7·3–7·6	10	30·0	14	21·4	72	15·3
Over 7·6	5	40·0	8	37·5	22	27·1

Table IV. Colpidium colpoda. *Effect of* p*H values.*

	Section 1		Section 2		Sections 5 and 6	
*p*H range	No. of cases	% occurrence	No. of cases	% occurrence	No. of cases	% occurrence
Under 4·9	36	38·9	8	50·0	—	—
4·9–5·2	10	50·0	6	50·0	—	—
5·3–5·6	72	68·1	21	47·6	6	50·0
5·7–6·0	34	67·6	30	83·3	3	66·6
6·1–6·4	25	84·0	34	73·5	21	81·1
6·5–6·8	86	86·1	112	58·1	66	19·7
6·9–7·2	40	90·0	70	72·8	150	48·0
7·3–7·6	13	69·3	14	100·0	71	26·7
Over 7·6	5	100·0	8	100·0	22	68·2

Table V. Paramoecium putrinum. *Effect of* p*H values.*

	Section 1		Section 2		Sections 5 and 6	
*p*H range	No. of cases	% occurrence	No. of cases	% occurrence	No. of cases	% occurrence
Under 4·9	36	25·0	8	37·5	—	—
4·9–5·2	12	33·3	6	66·6	—	—
5·3–5·6	29	41·8	19	26·3	6	33·3
5·7–6·0	38	50·0	40	57·5	3	100·0
6·1–6·4	26	61·6	33	75·8	21	28·5
6·5–6·8	90	83·3	111	81·1	64	65·5
6·9–7·2	52	82·7	63	68·3	147	74·4
7·3–7·6	12	66·7	14	71·5	71	63·4
Over 7·6	5	80·0	8	75·0	22	63·1

Table VI. Pleuronema chrysalis. *Effect of* p*H values.*

	Section 1		Section 2		Sections 5 and 6	
*p*H range	No. of cases	% occurrence	No. of cases	% occurrence	No. of cases	% occurrence
Under 4·9	32	0	8	12·5	—	—
4·9–5·2	12	8·5	6	0	—	—
5·3–5·6	27	25·9	19	5·2	6	0
5·7–6·0	39	20·5	40	32·5	3	0
6·1–6·4	30	53·3	34	47·1	21	23·9
6·5–6·8	98	77·5	112	78·6	65	23·1
6·9–7·2	52	88·0	68	66·2	149	55·0
7·3–7·6	10	50·0	14	100·0	70	61·4
Over 7·6	5	60·0	8	25·0	22	18·1

Table VII. Arcella vulgaris. *Effect of* p*H values.*

	Section 1		Section 2		Sections 5 and 6	
*p*H range	No. of cases	% occurrence	No. of cases	% occurrence	No. of cases	% occurrence
Under 4·9	1	0	2	100·0	—	—
4·9–5·2	3	33·3	1	0	—	—
5·3–5·6	13	15·4	17	23·5	2	100·0
5·7–6·0	32	9·4	29	31·1	7	100·0
6·1–6·4	36	13·9	29	34·5	33	89·9
6·5–6·8	41	9·7	52	7·7	98	68·4
6·9–7·2	62	14·5	52	9·6	168	71·5
7·3–7·6	2	0	3	0	13	69·3
Over 7·6	0	0	0	0	0	0

Table VIII. Cinetochilum margaritaceum. *Effect of* p*H values.*

	Section 1		Section 2		Sections 5 and 6	
*p*H range	No. of cases	% occurrence	No. of cases	% occurrence	No. of cases	% occurrence
Under 4·9	2	0	4	0	—	—
4·9–5·2	2	0	3	0	—	—
5·3–5·6	15	0	17	5·9	2	0
5·7–6·0	32	9·4	32	21·8	7	28·5
6·1–6·4	34	23·5	31	35·5	34	55·8
6·5–6·8	43	27·9	45	22·2	98	70·5
6·9–7·2	34	29·4	42	28·6	136	65·5
7·3–7·6	0	0	3	33·3	4	50·0
Over 7·6	0	0	0	0	0	0

Table IX. Bodo saltans. *Effect of* p*H values.*

	Section 1		Section 2		Sections 5 and 6	
*p*H range	No. of cases	% occurrence	No. of cases	% occurrence	No. of cases	% occurrence
Under 4·9	36	5·5	8	25·0	—	—
4·9–5·2	11	54·5	5	20·0	—	—
5·3–5·6	25	56·0	20	60·0	16	33·3
5·7–6·0	29	27·6	38	65·8	3	33·3
6·1–6·4	23	43·5	39	43·5	21	28·5
6·5–6·8	72	52·8	121	30·6	66	27·2
6·9–7·2	43	74·4	73	28·8	149	24·8
7·3–7·6	9	55·5	14	21·4	62	32·2
Over 7·6	5	80·0	8	75·0	22	91·0

Table X. *Effect of the omission of phosphate.*

		Percentage occurrences	
Species	Total No. of observations. Sections 1–5	Normal diet	No phosphate
Colpidium	55	19·8	73·3
Paramoecium	55	61·2	48·6
Cinetochilum	55	45·0	59·4
Pleuronema	55	50·4	43·2
Bodo	55	52·2	14·4
Arcella	55	50·4	32·4
Trepomonas	55	41·4	28·8

Table XI. *Effect of urea.*

Species	Total No. of observations. Sections 1–6	Percentage occurrences	
		No urea	Urea
Colpidium	148	19·4	25·5
Paramoecium	148	79·8	63·0
Cinetochilum	148	51·6	42·9
Pleuronema	148	54·9	74·4
Arcella	148	57·0	40·9

Table XII. *Effect of lactic acid.*

Species	Total No. of observations	Percentage occurrences					
		Before lactic. Sections		Lactic. Sections		After lactic. Sections	
		1 2 3	4 5 6	1 2 3	4 5 6	1 2 3	4 5 6
Bodo	36	39·2	8·4	5·6	2·8	0	5·6
Trepomonas	36	58·3	2·8	25·2	2·8	14·0	0
Colpidium	36	81·2	25·2	0	0	2·8	0
Paramoecium	36	98·0	100·0	70·0	98·0	100·0	75·5
Pleuronema	36	95·2	42·0	33·6	22·4	92·4	11·2
Lionotus	36	30·8	84·0	2·8	5·6	47·5	2·8

consideration to some extent, therefore as might be expected the top section during the lactic acid period was almost devoid of living Protozoa, though bacteria and fungi were present and good purification was ultimately obtained. The fact that the percentage purification brought about by the top section changed from 17 on the second day of the experiment to 58 on the fourteenth day suggests that the bacterial population also underwent a marked change, strains presumably being selected out from the normal population which were able to oxidise the acid, while the development of the sucrose splitting strains was inhibited. Table XII shows the percentage occurrences of each species in the top and bottom parts of the filter during the lactic acid period, when observations were made during 12 days, and also during the 12 days immediately preceding and following this period when the food supply was normal. It will be seen that *Paramoecium* shows no change in numbers except in the top half of the filter during the acid period, the diminution being entirely due to the absence of this organism from the top section (average *p*H 4·0). *Pleuronema* was depressed throughout the acid period but recovered as soon as normal food was resumed; the other four species considered not only were very much reduced during the acid period, but showed little or no recovery in the succeeding period, except in the case of *Lionotus* when there was a definite rise in numbers. In the case of the other species no recovery took place, and they were not seen until the gravel of the filter had been autoclaved and reinoculated. Reinoculation of the sections before sterilisation had no effect. These results suggest that in these species it is the flora encouraged by the lactic acid that is inimical, while in the case of *Pleuronema* and still more in that of *Lionotus* it is possibly the oxidation products of the acid that inhibit development.

IV. ACKNOWLEDGMENT.

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V. SUMMARY.

1. The purity of a medium, as measured by the amount of reducing material present in the solution, and the food supply, are two of the principal factors influencing the distribution of Protozoa in sewage filters.

2. The Protozoa considered occur throughout a wide range of pH values, but the optima for different species are different.

3. Where chemical compounds added to the solution affect the protozoan population adversely, it may be due either to the formation of deleterious oxidation products, or to the development of a bacterial flora which is inimical to the Protozoa.

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THE MICROBIOLOGY OF FARMYARD MANURE DECOMPOSITION IN SOIL.

I. CHANGES IN THE MICROFLORA, AND THEIR RELATION TO NITRIFICATION.

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(With Thirteen Text-figures.)

Introductory.

It is a well-known fact that farmyard manure, the oldest and most widely used nitrogenous fertiliser, shows a fertilising effect which is much smaller than should correspond to its total content of N, if this were fully available to the plants. The continuous wheat plots on Broadbalk, Rothamsted, the oldest field experiments dealing with this subject, have shown a utilisation by the wheat plants of 26 per cent. of the total amount of N added as farmyard manure over the period 1865 to 1912 (Russell (66)). Other investigators also give figures which, though variable, show incomplete utilisation of the manure N, as shown below.

Character of experiment	% of manure N utilised	Author
4 years field experiments	7–46	Schultze*
3 ,, ,,	32–51	Welbel*
2 ,, ,,	28–34	Schneidewind*
3 ,, pot experiments	31–41	Pfeiffer (56)
3 ,, field experiments	92–93	
2 ,, pot experiments	0–40	Löhnis and Smith (42)
10 ,, field experiments	15	Lipman and Blair (39)
1 year pot experiments	3–31	Goeters (21)
1 ,, ,,	13–24	Glathe (20)
1 ,, ,,	4–18	Gerlach and Seidel (19)
1 ,, field experiments	8–17	
2 years cylinder experiments	About 30	Balks (4) and Bach (3)
8 ,, field experiments	28–68	Iversen (29)

* Cit. after Löhnis (40).

This incomplete uptake of manure N by the plant is associated with its incomplete nitrification in soil. Tuxen (75) found only 33 per cent. of the N of farmyard manure nitrified in loam soil after 5 months, and after 15 months there was no appreciable change; in a sandy soil only 10 per cent. was nitrified after 10 months, but 56–74 per cent. after 13–15 months. In the same soils bone meal and guano were nitrified to an extent of 40–72 per cent. after 1–3 months. Dehérain (13) found 15–27 per cent. of the N of various samples of farmyard manure leached out of unplanted soil as nitrate during one year. Wagner (77) found that in a 522 days' laboratory experiment 39–40 per cent. of manure N was nitrified, whereas blood meal and lucerne showed a nitrification of 73–75 per cent. Popp (58) found 33–34 per cent. of manure N nitrified after 6 weeks, but no considerable further nitrification after 12 weeks. Löhnis and Smith (42) found that variously treated samples of manure were nitrified to an extent of 18–50 per cent. in 6–12 weeks, the nitrification being best where urine had been added to the manure. The most complete series of experiments in this direction has been carried out by Barthel and Bengtsson (5, 6), who arrived at the final conclusion that only the ammonia N of well-fermented manure will undergo any nitrification during the first 12–14 months in soils of different character with or without addition of lime. This interesting result would fully explain the slow fertilising action and incomplete nitrification of manure N. Somewhat different results, however, have been obtained by Sebelien (71), who found that 30–40 per cent. of the N of urine-free faeces of cattle and horses was nitrified in moist soil within 6 months, and by the writer (30), who found a nitrification of about 25 per cent. of the N of fresh, ammonia-free manure in the same time. Also Glathe (20), Goeters (21) and Scheibe (69) found in several instances a nitrification of more N than corresponded to the content of ammonia in the manure. The reservation should here be made that none of these experiments has been carried out with manure of quite the same kind as that used by Barthel and Bengtsson—ordinary, well-fermented and decomposed farmyard manure.

We find an analogy to the incomplete nitrification of manure N in the case of other organic N compounds, such as bone meal, guano, blood meal and lucerne as mentioned above. There is further evidence in the literature (Withers and Fraps (88), Popp (58), Wright (90), Hill (24), Lathrop (34), Honcamp (27), Martin (47), Holtz and Singleton (26), Jensen (30)) to show that nitrogenous organic compounds, when added to the soil, undergo a more or less rapid nitrification (sometimes preceded by a temporary depression of nitrate formation) which gradually slows down and tends to come to a standstill before all N has been nitrified. This gradual slowing down of the process of decomposition was observed as early as 1886 by Wollny (89), who enunciated the principle that the further a substance has been decomposed, the more slowly does the decomposition of the residue proceed. There seem to be very few exceptions to this rule of incomplete nitrification. Whiting and Richmond (84) found that leaves of sweet clover were completely nitrified after 3 months with an apparent stimulation of the nitrification of the soil's own N after 8 months but, unfortunately, it is not clear from their data whether the nitrate formed from the soil organic matter itself has been taken into account. Löhnis (43) found, in a very interesting series of experiments, that an addition of small amounts of young plant material had a tendency to stimulate the decomposition of humus in a fertile soil. It would *a priori* be expected that a certain proportion of the organic N would remain undecomposed as microbial substance. This has been suggested by Ramann (61): "Da die Zerstörung jedoch niemals vollständig wird, sondern mindestens die Leiber der zuletzt tätigen Organismen übrig bleiben müssen, verläuft der Vorgang asymptotisch, er nähert sich dem Nullpunkt ohne ihn doch jemals zu erreichen." The residue of N, which remains as microbial substance, is obviously greater, the more the energy material supplied in proportion to the amount of N. This accounts for the harmful influence of straw on the action of manure (Krüger and Schneidewind (33), v. May (48), Hansen (23), Niklewsky (52)), which was first ascribed to denitrification, but which has later, perhaps in a somewhat one-sided way, been explained exclusively as due to the formation of microbial protoplasm (Pfeiffer and Lemmermann (57), Murray (50), Rahn (60), Allison (1)). This leads us to the question of the importance of the C:N ratio, which was first systematically studied by Doryland (15), although attention had been called to it before by Löhnis (40), and which has recently been discussed in detail especially by Waksman and his co-workers (79, 83). Farmyard manure is an organic material of a rather low N content (C:N–15–20:1), from which we would expect a rather slow and incomplete nitrification, such as is found in experiments. One would also expect a very large accumulation of organic N compounds in continuously manured soils, owing to the incomplete utilisation of the N by the plants. This is the case to a certain extent only. Tuxen (76) found, in soils fertilised for from 22 and 30 years, a very notable increase in N due to farmyard manure, but very little due to artificial fertilisers. The experiments on Broadbalk (Russell (66)) show that during 47 years only about 14 per cent. of the N of the added manure has been accumulated in humus; about 25 per cent. has been utilised by the plants, and the remaining 60 per cent. appears to be lost, probably as gaseous N, since there is very little drainage from the field. Similar losses of manure N have been recorded in America by Lipman and Blair (39), from whose data it is seen that about 55 per cent. manure N has been stored in the soil and about 30 per cent. lost, and in Denmark by Christensen (9), who found in soils from fertilising experiments recorded by Iversen (29) a N storage (expressed as excess in N of farmyard manured plots over artificially fertilised plots) of 14–17 per cent. of the total amount of N supplied as farmyard manure during 8 years; 28 to 68 per cent. of the N had been utilised by the plants, so that here also there is a notable loss of N. Whether this is due to leaching is uncertain, since no analyses of drainage water were made.

The present work was designed to give some information on the following questions:

I. Is the relatively low nitrification of the organic N of farmyard manure a general rule?

II. Is a part of the N of the manure in itself inaccessible to the attack of the soil micro-organisms, or can the incomplete nitrification and utilisation of the manured N by the plants be explained through the C:N ratio of the manure, *i.e.* the synthesis of new microbial protoplasm?

III. Does a loss of manure N occur in laboratory experiments under conditions where leaching is excluded?

The following sets of experiments were carried out:

1. Various kinds of farmyard manure were allowed to decompose in soils of different character, and the development of bacteria, actinomycetes and fungi was compared with the formation of nitrate and the disappearance of carbon. Amounts of "humic acid" and possible losses of total N were estimated.

2. The nature of the micro-organisms decomposing the cellulosic material of the manure in the soil was studied, and the organisms were tested for their nitrogen requirements and their ability to decompose lignified cellulose.

3. Various kinds of microbial protoplasm were submitted to decomposition experiments in soil and sand, and in order to ascertain firstly whether the hypothesis that bacterial matter is particularly resistant to decomposition is tenable, and secondly whether resistant, humus-like nitrogenous compounds are of common occurrence in the protoplasm of micro-organisms.

Experiments falling in the first section are described in the present paper. Later contributions will deal with the two others.

THE GENERAL ASPECT OF THE SOIL MICROFLORA DURING DECOMPOSITION OF MANURE, AND ITS RELATION TO NITRIFICATION.

(1) *Technique.*

The decomposition experiments described below were carried out in the same manner as those of Barthel and Bengtsson: soil with addition of manure or other organic material, as well as control soil without addition, was kept in wide-mouthed glass bottles, closed with tight-fitting corks, with holes through which passed glass tubes approximately 1 cm. wide filled with cotton-wool; this arrangement admits the air fully and reduces the evaporation of moisture to a minimum. The bottles were of such a size that the diameter and the depth of the soil mass were about equal at the start of the experiment. 1·2–1·5 kg. of soil was used for the long-period experiments. Samples were taken, each time after careful mixing, at various periods of time, and estimations were made of the numbers of bacteria, actinomycetes and fungi, amounts of NO_3-N and NH_4-N, and in some cases, especially at start and end of experiments, of total N, total carbon, α-humus, and its N content. The numbers of micro-organisms were calculated on the basis of fresh, moist soil, while the soil samples for chemical determinations were dried overnight at about 35° C. and the result calculated on the basis of air-dry soil.

The following methods were used:

Total nitrogen was determined by the routine Kjeldahl method; when soils containing considerable amounts of nitrate were to be analysed, the method for including nitrate (digestion with phenoldisulphonic acid and reduction with sodium thiosulphate) was used.

Nitrate nitrogen was determined by the Devarda method.

Ammonium nitrogen was determined by Bengtsson's KCl method: repeated extraction of the soil with 0·5 per cent. KCl solution and distillation of the extract with magnesium oxide.

Total carbon was determined by the simplified combustion method of Dennstedt (14).

Humus (α-humus, crude mixture of humic and hymatomelanic acids) was determined by the method of Waksman (81): twice repeated extraction of the soil with 2·5 per cent. NaOH solution by heating for 30 minutes in the autoclave at 15 lb. pressure; the solution is filtered off, the soil is washed with NaOH solution and water, and the humus is precipitated from the extract with HCl; after sedimentation the precipitate is filtered off on weighed filter-paper, washed and dried for 20–24 hours at 55° C.

Numbers of bacteria and actinomycetes were determined by the plate method: 10 gm. of moist soil were shaken for 4 minutes with 250 c.c. of a solution of 0·5 per cent. NaCl and 0·05 per cent. $MgSO_4$, and from a suspension diluted to 1:250,000, five or six parallel plates were poured and incubated for 10 days at 20° C. Two different agar media were at first compared. The first was the mannite-asparagine agar generally used for counting soil bacteria in this laboratory (Thornton (73)). This medium has been found by Fisher, Thornton and Mackenzie (16) to furnish counts which are in agreement with theoretical requirements, *i.e.* the numbers of colonies appearing on the plates depend only on the numbers of bacterial cells in the inoculum. The second medium was a modification of the albumen agar recommended by Waksman (78): dextrose 2·0 gm., casein, dissolved in 0·1N NaOH, 0·2 gm., K_2HPO_4 0·5 gm., $MgSO_4$ 0·2 gm., agar 15 gm., H_2O 1000 c.c., pH 6·5–6·6. This medium had previously been used by the writer in making counts from several Danish soils (see Christensen (9)), and since it had been found then to furnish good results, it was thought worth while to compare it with the mannite agar, the only medium for which the index of dispersion $\chi^2 = \frac{S(x - \bar{x})^2}{\bar{x}}$ (Fisher (17)) has yet been calculated. The results showed that the casein agar gave as good a distribution of the χ^2 values as the mannite agar (especially for the actinomycetes counts which showed subnormal variation on mannite agar), and since the casein medium gave constantly higher counts, it was afterwards used exclusively.

Counts of fungi were made by the method suggested by Brierley *et al.* (8): soil suspension was shaken as uniformly as possible for 20 minutes, using flasks of similar shape and size in all cases. Suspension diluted to 1:1000 (or a higher dilution for soils particularly rich in fungi) was plated out on Conn's glycerin agar of pH 4·6–4·8. Six parallel plates were incubated for 7 days at 25° C.

(2) *First series of experiments.*

In this series the decomposition of manure alone and mixed with straw was studied in acid and neutral soil. Two soils were used, both from the Rothamsted Grass plots; they were heavy clay soils, rich in organic matter. The first was from unmanured plot No. 1 of pH 7·0, the second from plot No. 14A (unlimed, mineral fertiliser + $(NH_4)_2SO_4$) of pH 3·8. The air-dried soils received 30 and 33 per cent. H_2O, and further, on air-dry basis:

(1) No addition (control soil).
(2) Farmyard manure corresponding to 2 per cent. dry matter.
(3) Same + 2 per cent. ground oat straw.

The manure was old, well decomposed and humified, but contained, probably owing to bad storage, very little soluble nitrogen. The composition of the manure and of the straw were:

	Manure	Straw
Dry matter (%)	28·0	92·3
Total N, % of dry matter	1·96	0·52
NH_4-N	0·10	—
Total C	29·2	43·3
Ash	40·6	5·7
α-Humus	14·1	—
% N content of α-humus	2·57	—

The experiments were run at room temperature for 500 days, and complete analyses were made after 300 days and at the end. The counts of micro-organisms are seen in Figs. 1–2 and the chemical changes in Tables I–II.

In the neutral control soil the numbers of bacteria did not undergo any very marked changes beyond the fluctuations which these figures normally show, especially in air-dried and re-moistened soil. The marked fall in bacterial numbers in soils kept in bottles in the laboratory, which has been observed by Cutler and Dixon (10), did not appear in these counts on casein agar, but was very conspicuous in counts on mannite agar after 100–125 days. In the soil + manure there was a very great increase in the numbers of bacteria after 10 days, but the numbers fell as rapidly and slowly approached those in the control soil, so that after 150 days the figures from the two soils showed no significant differences. In the soil with manure + straw there was, as might be expected, an enormous increase in bacterial numbers. This increase lasted longer, but the general trend of the figures was the same as in the previous soil: the increase was followed by a decrease which was rapid at first, then slower, so that the numbers gradually approached those of the control soil and eventually reached them, although the influence of the straw was still quite noticeable after 200–250 days. The actinomycetes behaved similarly to the bacteria, save that the changes in their numbers were less pronounced and that their numbers tended to become relatively higher with advancing time. The fungi were not at all affected by the addition of manure, but the extra addition of straw caused a very great increase in their numbers, an increase which was probably due to vegetative growth and not merely to sporulation, since it was, like that of the bacteria, only temporary. A high count of fungi, due to resting spores, would probably remain constant for a much longer period. The increase was almost entirely due to one particular fungus (*Cephalosporium* sp.)

which probably utilised the sugars and pentosans of the straw, since it did not grow on filter paper cellulose in pure culture.

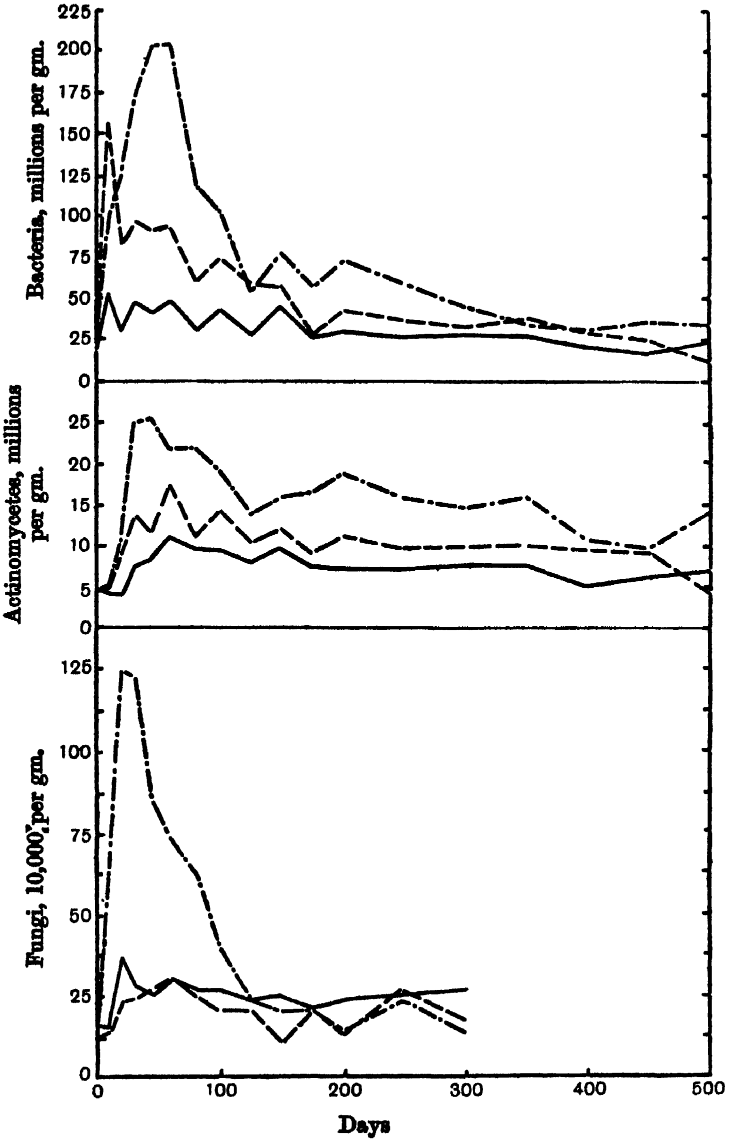

Fig. 1. Numbers of micro-organisms in neutral Park plot soil.

——— Control soil. – – – – Soil + manure. —·—·— Soil + manure + straw.

In the acid soil, conditions were very different. The bacterial numbers were very low and the addition of manure and straw did not give rise to any considerable multiplication of bacteria or actinomycetes. It is, of course, conceivable that such organisms, specially adapted for the acid conditions and not capable of developing on the neutral agar medium, might have been active here; however, no striking development of such organisms took place on the acid agar used for counting fungi. The fungi were only slightly stimulated by the addition of manure, but in the soil with manure + straw their numbers at once rose to enormous heights and *remained constant* for a very long period. The most strongly multiplying forms were: *Trichoderma* sp. (Koningi?), which is an active cellulose-decomposing organism, *Zygorhynchus* sp. (Vuilleminii?), and a little yellow fungus, probably an *Amblyosporium*. When these experiments had run for some time there appeared a paper by McLennan (49), who developed a method for distinguishing between fungus spores and mycelium in the soil by drying the soil in vacuum; this treatment kills the vegetative mycelium, but leaves the spores intact. A count by this

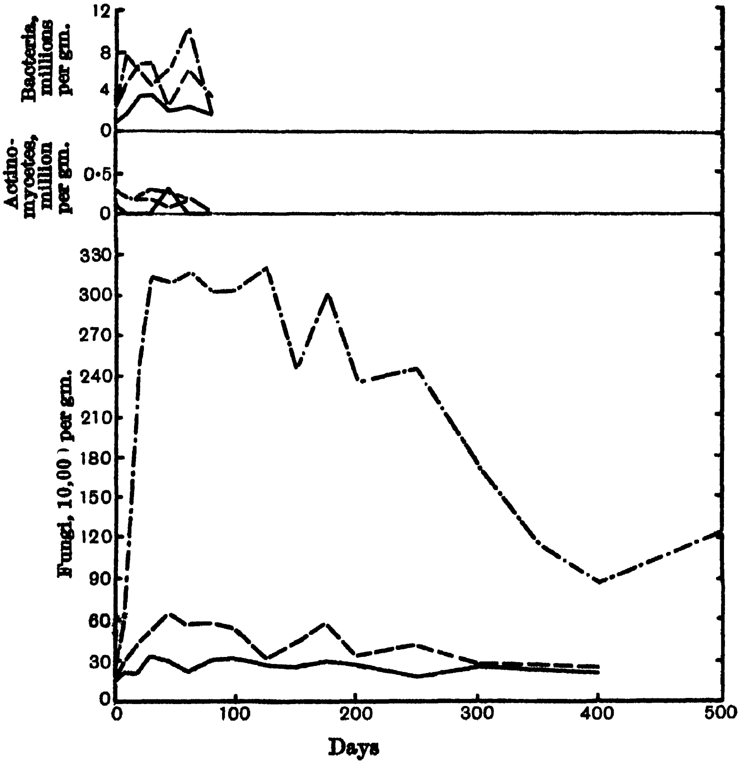

Fig. 2. Numbers of micro-organisms in acid Park plot soil.
——— Control soil. – – – – Soil + manure. —·—·— Soil + manure + straw.

method was carried out in these three soils after 400 days with the following result:

Soil	Fungi, thousands per gm. Fresh	Fungi, thousands per gm. Dried *in vacuo*	Remarks
Control	200	80	No *Trichoderma* in dried soil
Soil + manure	240	104	Very few *Trichoderma* in dried soil
Soil + manure + straw	880	380	Very few *Trichoderma* in dried soil

The fungus colonies on the plates apparently originated from spores as well as from vegetative mycelium, but the *Trichoderma* (and *Zygorhynchus*) seem to be present mainly as mycelium, since they were most

Table I. *Chemical changes in Park plot soil, pH* 7·0, *with addition of old farmyard manure and straw.*

	Control soil		Soil + manure		Soil + manure + straw	
Time	NO_3-N*	NH_4-N*	NO_3-N	NH_4-N	NO_3-N	NH_4-N
Start	0·0	0·4	0·0	1·0	0·0	1·0
After 100 days	0·8	0·0	0·8	0·0	1·2	0·0
„ 150 „	6·0	0·0	12·6	0·0	5·1	0·0
„ 200 „	8·4	0·0	14·0	0·0	2·4	0·0
„ 250 „	10·9	0·0	20·9	0·0	8·5	0·0
„ 300 „	13·3	0·0	24·9	0·0	12·8	0·0
„ 350 „	17·4	0·0	29·5	0·0	19·2	0·0
„ 400 „	18·9	0·0	31·1	0·0	21·5	0·0
„ 450 „	20·2	0·0	30·2	0·0	24·4	0·0
„ 500 „	19·9	0·0	32·5	0·0	24·7	0·0
Excess of NO_3-N over control, as % of added N	—		29·4		0·0	
	—		32·1		10·2	
α-humus as % of total dry matter:						
Start	2·19 with 4·11 N		2·47 with 3·93 N		2·38 with 4·08 N	
After 300 days	2·06 „ 4·00 N		2·43 „ 3·63 N		2·53 „ 3·51 N	
„ 500 „	1·81 „ 3·77 N		2·36 „ 3·38 N		2·53 „ 3·15 N	
Excess of α-humus N over control, as % of added N	—		18·1		14·5	
	—		14·5		14·1	
	—		30·6		24·2	
% total N:						
Start	0·355		0·394		0·404	
After 300 days	0·361		0·402		0·403	
„ 500 „	0·360		0·390		0·389	
% total C:						
Start	4·37		4·95		5·76	
After 300 days	4·01		4·42		4·67	
C : N ratio†:						
Start	12·3 : 1		12·6 : 1		14·3 : 1	
After 300 days	11·5 : 1		11·7 : 1		12·0 : 1	

* mg. of N per 100 gm. of air-dry soil.
† Mineral N subtracted.

strongly affected by the drying. The higher counts thus do not merely indicate that spore formation has taken place, but that there has been an active growth of fungi, resulting in a formation of considerable amounts of mycelium which is still living after 400 days.

The chemical changes taking place in the soils are shown in Tables I–II. The figures show that the accumulation of nitrate in the neutral control soil was very small for the first 100 days, probably because the soil was rich in grass roots and other plant residue poor in N. But in the interval from 100 to 150 days the nitrate production became active and proceeded regularly, gradually becoming slower, so that after 400–500 days about 200 parts per million of nitrate N accumulated. The soil + manure behaved somewhat similarly: no nitrate accumulated during the first 100 days, but from then the nitrate production proceeded fairly regularly until after 350 days, from which time very little more nitrate accumulated. At the end, the excess of nitrate over control corresponded to 32·1 per cent. of the manure N. In the soil with manure + straw the depression of nitrate formation was of course very marked, but wore off gradually, so that after 300 days the nitrate content about reached the level of the control soil. By this time the C:N ratio, originally 14·8:1, had been narrowed to 12·0:1, which is nearly the same as in the other two soils. From now on nitrate was produced in excess over control soil, but the nitrification remained less complete than in the case of manure alone. After 300 days the soil + straw had lost considerably more of its carbon than the other two soils, suggesting that the carbon of the straw is more easily attacked than that of the manure, as also found by Lemmermann *et al.* (35), Fraps (18), Potter and Snyder (59), and Lemmermann and Wiessmann (36). The soils thus gradually adjust themselves to the same C:N ratio, similar to that generally found in field soils. The total N determinations during the course of the experiment did not show any changes of undoubted significance. The humus determinations show that the amount of α-humus decreased slowly in the control soil, and that the N percentage of what remained fell from 4·11 to 3·77. In the soil + manure the amount of humus also decreased a little during the experiment, but remained constantly in excess over that of the control soil, and this excess was somewhat larger than the approximate 0·3 per cent. introduced with the manure. This suggests that either the added organic matter in these soils had protected the soil humus from attack, or else that some additional humus had been synthesised here. The same is the case in the soil with manure + straw, where the introduction of lignin with the straw further increased the amount of humus a little. The percentages of N in humus in the two treated soils were lower than in the control soil, because the humus of the manure contains only 2·6 per cent. N. In these soils, as in the control, the N content of the humus fell during the storage, but by the end of the experiment the total humus N in the manured soil still exceeded that of the control soil by an amount equivalent to about 30 per cent. of the manure N added.

Table II. *Chemical changes in Park plot soil, pH* 3·8, *with addition of old farmyard manure and straw.*

	Control soil		Soil + manure		Soil + manure + straw	
Time	NO_3-N*	NH_4-N*	NO_3-N	NH_4-N	NO_3-N	NH_4-N
After 100 days	1·1	7·9	2·6	8·8	0·5	0·0
„ 150 „	0·3	7·8	2·1	8·1	0·0	1·4
„ 200 „	0·7	9·2	3·9	11·9	0·5	4·2
„ 250 „	0·0	12·8	1·4	16·0	0·0	6·6
„ 300 „	0·2	18·5	2·4	22·0	Trace	14·2
„ 350 „	0·2	18·7	1·4	24·2	0·0	15·6
„ 400 „	1·4	20·3	2·3	24·1	1·6	16·2
„ 500 „	1·9	18·2	2·2	24·9	1·7	17·7
Final excess of mineral N over control, as % of added N	—		17·8		0·0	
a-humus %:						
After 300 days	4·72		4·98		5·33	
% N in a-humus:						
After 300 days	3·37		3·30		3·15	
Final excess of a-humus N over control, as % of added N	—		13·3		18·0	

* mg. per 100 gm. of air-dry soil.

In the acid soils no determinations of ammonia and nitrate were made at the start of the experiment. From 100 days and onwards there was an accumulation of considerable amounts of ammonia and small amounts of nitrate in the control soil and the soil + manure, but the ammonification of the manure here proceeded much more slowly than its nitrification in the neutral soil, so that after 500 days the excess of ammonia + nitrate over control corresponded to only 17·8 per cent. of the manure N. In the soil with manure + straw the depression in the formation of mineral N was still more pronounced than in the neutral soil, as might be expected from the abundant development of fungi, but in this soil also the depression gradually wore off, and after 500 days the content of ammonia was nearly on a level with that of the control soil. In these soils too the humus of the manure persisted, and the addition of straw gave a marked increase in humus content after 300 days, probably because the lignins did not undergo any noticeable decomposition in this extremely acid soil. It is noteworthy that in the third soil the excess in humus over soil + manure was 0·3 per cent., and the addition of 2 per cent. straw corresponds to an addition of approximately 0·4 per cent. lignin.

(3) *Second series of experiments.*

In this series the decomposition of fresh farmyard manure with and without extra addition of ammonium sulphate and straw in faintly acid soil with and without lime was studied.

The soil used for this experiment was a light sandy soil, rather poor in organic matter and of *p*H 6·0 from Woburn Experimental Station. The air-dry soil received 16 per cent. water and 0·2 per cent. $Ca(H_2PO_4)_2$ in order to make its reaction a little more acid. The soil contained at the start of the experiment (on air-dry basis): total C, 1·22 per cent.; total N, 0·112 per cent.; α-humus, 0·67 per cent. with 3·8 per cent. N. The following experiments were started:

(1) No addition (control)
(2) Manure corresponding to 2 per cent. dry matter
(3) Do. + 0·0538 per cent. $(NH_4)_2SO_4$
(4) Do. + Do. + 0·7 per cent. oat straw

$\Big\}$ All in two separate series with and without 1 per cent. $CaCO_3$

The manure was from a manure heap left loosely in the open. It contained 17·6 per cent. dry matter and 0·349 per cent. N, of which only a very small part was present as ammonia. The composition of the manure was on a basis of dry matter:

Total N	1·93 %	**Ash**	27·0 %
NH_4-N	0·06	**α-humus**	16·5
Total C	39·6	**N in α-humus**	2·2

The C:N ratio of the manure was thus 20·6:1. The addition of ammonium sulphate gave the manure a content of 2·50 per cent. N, corresponding to a C:N ratio of 15·8:1, and the further addition of straw with 0·48 per cent. N and 40·1 per cent. C restored approximately the original C:N ratio—21·6:1.

The soils were kept for 360 days at room temperature. The counts of micro-organisms are seen in Figs. 3 and 4, and the chemical changes in Tables III and IV.

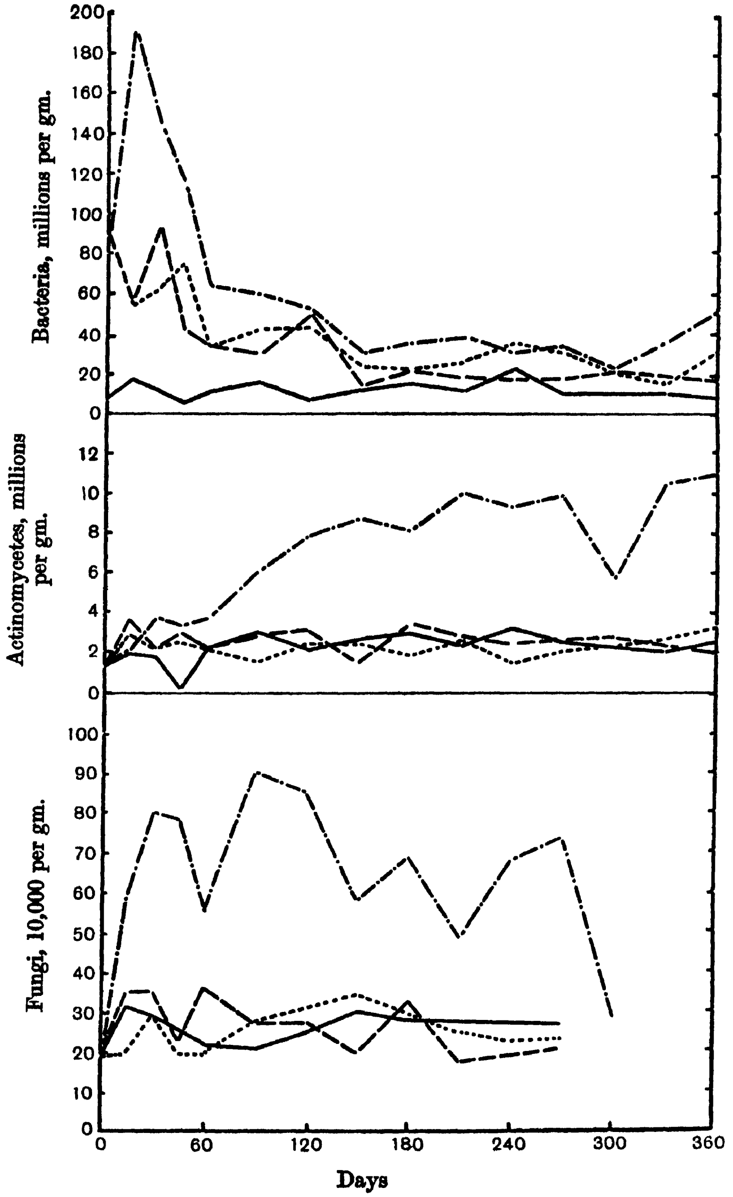

Fig. 3. Numbers of micro-organisms in Woburn soil, without $CaCO_3$.
——— Control soil. – – – – Soil + manure. ······ Soil + Do. + $(NH_4)_2SO_4$.
—·—·— Soil + Do. + Do. + straw.

In the control soils the numbers of micro-organisms were quite low in this case, and the liming had no effect. In the soils with additions of manure the initial figures were high, on account of the large numbers of living organisms introduced with the fresh manure. In the soils with manure alone and with manure + extra N the figures fell gradually from the start, and after 90–150 days became about equal to those of the control soils, but the extra addition of straw caused the bacteria to multiply vigorously both in limed and unlimed soil, and not until after 200 days did the numbers go down to about the same range as in the control soils. The actinomycetes were not much affected by manure or

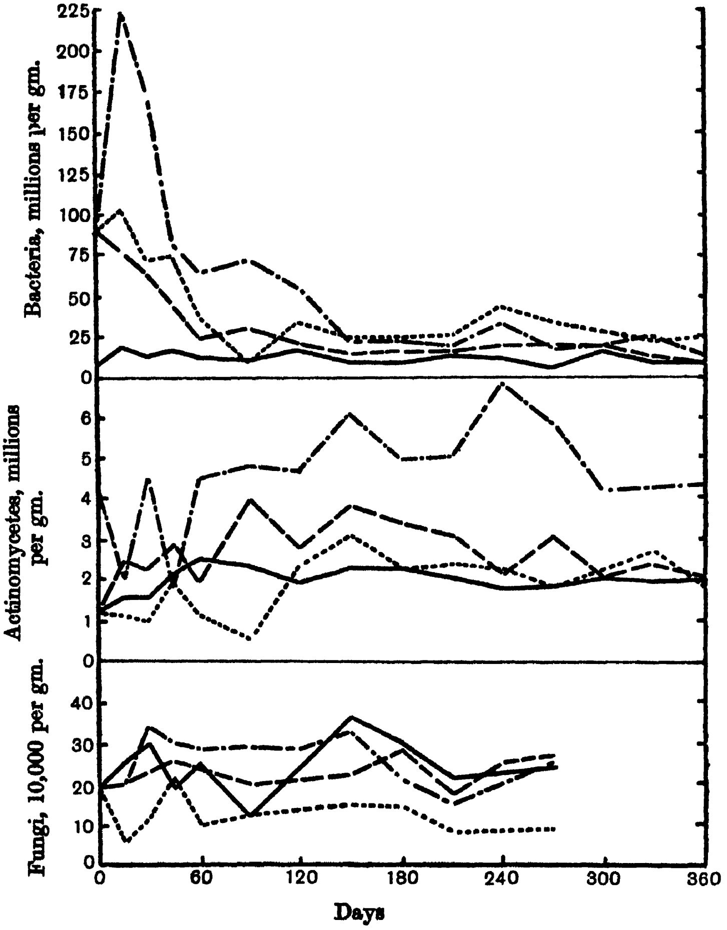

Fig. 4. Numbers of micro-organisms in Woburn soil + $CaCO_3$. Second series.
——— Control soil. – – – – Soil + manure. ······ Soil + Do. + $(NH_4)_2SO_4$.
—·—·— Soil + Do. + Do. + straw.

Table III. *Chemical changes in Woburn soil, unlimed, pH* 6·0, *with addition of farmyard manure, ammonium sulphate, and straw.*

	Control soil		Soil + manure		Soil + manure + $(NH_4)_2SO_4$		Soil + manure + $(NH_4)_2SO_4$ + straw	
Time	NO_3-N*	NH_4-N*	NO_3-N	NH_4-N	NO_3-N	NH_4-N	NO_3-N	NH_4-N
Start	3·5	0·2	3·5	0·4	3·5	11·8	3·5	11·8
After 45 days	5·4	0·0	8·1	0·1	17·3	0·0	9·7	0·3
„ 90 „	6·5	0·0	9·2	0·0	21·3	0·0	9·5	0·0
„ 120 „	6·8	0·0	10·0	0·0	22·0	0·0	11·4	0·0
„ 150 „	7·0	0·0	10·3	0·0	21·6	0·0	12·6	0·0
„ 180 „	7·5	0·0	11·9	0·0	24·4	0·0	15·6	0·0
„ 210 „	7·1	0·0	11·9	0·0	25·0	0·0	17·1	0·0
„ 240 „	9·9	0·0	17·8	0·0	28·4	0·0	17·7	0·0
„ 270 „	8·7	0·0	16·8	0·0	28·0	0·0	16·1	0·0
„ 300 „	7·9	0·0	14·6	0·0	29·2	0·0	16·8	0·0
„ 330 „	8·7	0·0	17·1	0·0	31·4	0·0	16·8	0·0
„ 360 „	8·7	0·0	17·3	0·0	29·4	0·0	18·6	0·0
Final excess of NO_3-N over control, as % of added N	—		22·3		41·4		18·5	
a-humus %								
Start:	0·67		1·00		1·00		1·00	
After 360 days	0·66		0·93		1·07		1·10	
% N in *a*-humus:								
After 360 days	3·63		3·22		3·30		3·40	
% total N:								
Start	0·112		0·148		0·159		0·162	
End	0·110		0·147		0·159		0·160	
% total C:								
Start	1·22		2·01		2·01		2·29	
End	1·22		1·76		1·83		1·88	
% added C disappeared	—		25·9		16·5		34·5	
Final C:N ratio†	12·4:1		13·5:1		14·1:1		13·3:1	
*p*H:								
After 90 days	5·7		5·8		5·5		5·7	
„ 360 „	5·6		5·6		5·4		5·6	

* mg. per 100 gm. of air-dry soil. † Mineral N subtracted.

$(NH_4)_2SO_4$, but were stimulated by the addition of straw, especially in the later stages of the experiment. As in the previous series of experiments, the fungi were not affected by the addition of manure or ammonium sulphate, except for the fact that their numbers seemed to drop markedly in the limed soil + ammonium sulphate. The addition of straw, however, caused them to multiply vigorously in the unlimed soil, and for a considerable period. After 300 days most of the fungus colonies on the plates originated from vegetative mycelium, as was shown by a count carried out according to the method of McLennan (49). This showed:

"Numbers" of fungi in fresh soil, per gm.	270,000
„ „ dried „	80,000

The increase in fungi in this soil was mostly due to one particular species, probably a *Monosporium*, which produced a vigorous growth on cellulose as filter-paper in mineral solution.

The chemical changes in this soil were very interesting. In the acid control soil the nitrate production followed a fairly smooth curve. In this soil with manure, nitrate was formed from the beginning, and, after 240 days, nitrate formation was marked, and subsequently maintained a fluctuating level so that at the end of the experiment the excess of nitrate over control corresponded to 22·3 per cent. of the N of the manure. In the soil with manure and ammonium sulphate the nitrification was stronger, and the excess over the previous soil was very nearly equal to the amount of N added as ammonium sulphate N. The curve ran very smoothly here, and the amounts of nitrate remained fairly constant after 240 days, so that at the end of the experiment the excess of nitrate over control corresponded to 41·4 per cent. of the total N added. This corresponds to all the added ammonium sulphate—24 per cent. of the manure N, almost the same as in the soil with manure alone.

The soil with manure + N + straw gave a nitrate content which, after 90 days and onwards, was almost the same as in the soil with manure alone, save that the rise occurred at a somewhat earlier date (180 days), and by the end of the experiment the nitrate corresponded to 18·5 per cent. of the added N. The fact that the C:N ratio of this material was somewhat higher than that of the manure alone perhaps explains the smaller percentage nitrification.

In the limed soils, the nitrate figures in the control soil were practically the same as those in the corresponding unlimed soil. The soil with manure showed a nitrate accumulation which, from 120 days onwards, was little different from that of the unlimed soil with manure (the figures fluctuated, but were on the same general level), excess of nitrate over control at the end of the experiment corresponding to 20·7 per cent. of the manure N. The soil with manure + N showed an increased nitrate accumulation which remained constant from 210 days onwards, but the increase was smaller than one would expect, and corresponded at the end of the experiment to only 26·8 per cent. of the total added N, or 6–7 per cent. of the manure N. Finally, in the soil with manure + N + straw the nitrate figures were almost identical with those from the soil + manure alone, so that at the end of the experiment the excess of nitrate over control corresponded to only 13·7 per cent. of the added nitrogen. The introduction of enough straw to restore the original C:N ratio was thus sufficient both in the unlimed and in the limed soil to prevent the nitrification of the added ammonium sulphate, but it is remarkable that

Table IV. *Chemical changes in Woburn soil, limed, with addition of farmyard manure, ammonium sulphate, and straw.*

Time	Control soil		Soil + manure		Soil + manure + $(NH_4)_2SO_4$		Soil + manure + $(NH_4)_2SO_4$ + straw	
	NO_3-N*	NH_4-N*	NO_3-N	NH_4-N	NO_3-N	NH_4-N	NO_3-N	NH_4-N
Start	3·5	0·2	3·5	0·4	3·5	11·8	3·5	11·8
After 45 days	5·9	0·0	7·0	0·2	16·9	0·0	9·0	0·0
" 90 "	6·7	0·0	6·0	0·0	17·3	0·0	8·7	0·0
" 120 "	7·0	0·0	9·2	0·0	19·9	0·0	9·5	0·0
" 150 "	7·1	0·0	11·6	0·0	19·8	0·0	11·3	0·0
" 180 "	7·2	0·0	12·3	0·0	21·4	0·0	13·9	0·0
" 210 "	7·4	0·0	14·8	0·0	22·0	0·0	15·6	0·0
" 240 "	7·8	0·0	14·2	0·0	28·0	0·0	14·6	0·0
" 270 "	8·5	0·0	16·5	0·0	22·1	0·0	16·0	0·0
" 300 "	8·4	0·0	19·4	0·0	22·4	0·0	17·1	0·0
" 330 "	8·4	0·0	17·2	0·0	23·0	0·0	17·1	0·0
" 360 "	8·7	0·0	16·7	0·0	22·1	0·0	16·0	0·0
Final excess of NO_3-N over control, as % of added N	—		20·7		26·8		13·7	
a humus %:								
Start	0·67		1·00		1·00		1·00	
After 360 days	0·53		1·02		0·85		0·93	
% N in *a*-humus:								
After 360 days	3·80		3·47		3·58		3·48	
% total N:								
Start	0·111		0·146		0·157		0·160	
End	0·102		0·144		0·147		0·141	
% total C (excl. of carbohydrates):								
Start	1·22		1·95		1·95		2·23	
End	1·22		1·74		1·62		1·73	
% added C disappeared	—		26·5		41·7		46·3	
Final C:N ratio†	13·2:1		13·7:1		12·9:1		13·8:1	
*p*H:								
After 90 days	6·7		6·7		6·5		6·6	
" 360 "	6·1		6·3		6·1		6·2	

* mg. per 100 gm. of air-dry soil.

† Mineral N subtracted.

the nitrification seemed less complete in the limed than in the unlimed soil. The determination of the total N at the end of the experiment suggests an explanation of this. In the acid soil series there were no significant changes in the N content, but the limed soil showed a loss which is considerable in the soil with manure + ammonium sulphate + straw. When we allow for this loss, we see that the amounts of N *left untransformed* were considerably larger in the acid soils. Thus we have here a real parallel to the losses of N in manured soils under field conditions: the drop in N content of the last soil—from 0·160 to 0·141 per cent.—corresponds to a loss of 35 per cent. of the added N during one year.

It is difficult to explain what may have been the cause of this loss, but it is not impossible that denitrification may have played a rôle, although the soil was but moderately moist and well aerated, and no large amounts of nitrate accumulated until the readily available energy material in the soil had been exhausted, as can be seen from the drops in the bacterial numbers. It must be remembered, however, that several experiments have shown that very slowly decomposible organic material (Nolte (53)) and soil humus (Oelsner (54)) may serve as sources of energy for denitrification, and experiments by Arndt (2) have shown a loss of N, probably due to denitrification, in limed high-moor soil which was well aerated and only moderately moist. This harmonises well with the fact that Winogradsky (86) has found obligate anaerobic *Clostridia* developing in their vegetative state in soil far from saturated with water. That the losses occur only in limed soils agrees with the fact that the denitrifying bacteria, as shown by Sacharowa (68), are very sensitive to acidity and stop their activity at *p*H 5·9–6·1.

A small additional experiment was carried out with the residue of unlimed soil + manure after the experiment had been discontinued. Two portions of 100 gm. of air-dry soil received each 17·0 per cent. water, one of them also 1 per cent. $CaCO_3$, and were kept at 25° C. for 25 days. Determinations of total N and nitrate N then showed:

	Soil without $CaCO_3$	Soil + $CaCO_3$
NO_3-N, mg. per 100 gm.	19·6	19·0
Total N, % at start	0·147	0·146
Total N, % at end	0·148	0·143

There was no significant change in the N content of either soil. An attempt was made to get an idea of whether there was a higher number of denitrifying bacteria in the latter soil. A dilution experiment in Giltay's solution was carried out with the following result (incubation for 7 days at 25° C.):

		Fermentation in solution inoculated with	
Dilution	Tube no.	Soil without $CaCO_3$	Soil with $CaCO_3$
1:100	*a*	+	+
	b	+	+
1:1000	*a*	+	+
	b	+	+
	c	−	+
	d	−	+
1:10,000	*a*	−	+
	b	−	−
	c	−	−
	d	−	−

There is an indication that denitrifying bacteria are a little more numerous in the limed soil, though the loss of N produced by them was insufficient to give a significant result by analysis. It would probably be worth while to undertake a closer study of the conditions under which denitrifying bacteria are able to cause a loss of N from the soil, for it seems possible that the importance of denitrification in the soil has been first exaggerated and then underestimated. It should be noted that the *p*H determinations in the soils show possibility of ammonia evaporation to be out of question.

The humus determinations from the Woburn soil show that the manured soils have 0·27–0·44 per cent. in excess over the control soils, and their humus is somewhat poorer in N than that of the control soils. The manure has originally introduced 0·33 per cent. humus with 2·2 per cent. N, which seems to have persisted fairly completely during the whole experiment. This amount has of course been further increased by the addition of lignin in the straw. The total C determinations show that, in the two control soils, the losses in C are within the limits of the analytical error—in accordance with the low numbers of micro-organisms in these soils—and the losses of manure C are not very large in the unlimed soils. The soils with addition of straw have lost most, as in the previous experiment. In the limed soils with manure and extra N the losses in C are considerable, so that, owing to the losses of N in the limed soils, the C:N ratios tend to become equal in all soils, namely 12·2–14·1:1, a somewhat wide ratio.

(4) *Third series of experiments.*

In this series a comparison between two manures poor and rich in nitrogen in heavy clay soil was studied.

In order to study the influence of the C:N ratio of the manure, an experiment was carried out with two manures, of which one was very poor, the other very rich in N. The soil was a heavy clay soil from Hoosfield, fairly rich in organic matter and of *p*H 6·3. It contained in air-dry condition: total N, 0·165 per cent.; total C, 1·84 per cent. The N-poor manure was the same as in the previous experiment, the N-rich, a sample of manure treated by the Edelmist process, which has in recent years attracted considerable attention in Germany[1]. It contained

Dry matter (%)	... ...	22·4
Total N, % of dry matter	... ...	3·39
NH_4-N „	... ...	0·34
Total C „	... ...	43·4
Ash „	... ...	20·5
α-humus „	... ...	27·5
N in α-humus „	... ...	2·97

---

[1] The manure was supplied through the courtesy of Gärstatt G. m. b. H., Munich, Germany.

The manures were used in the same quantities as before—corresponding to 2 per cent. of dry matter, and flasks were kept at room temperature for 360 days.

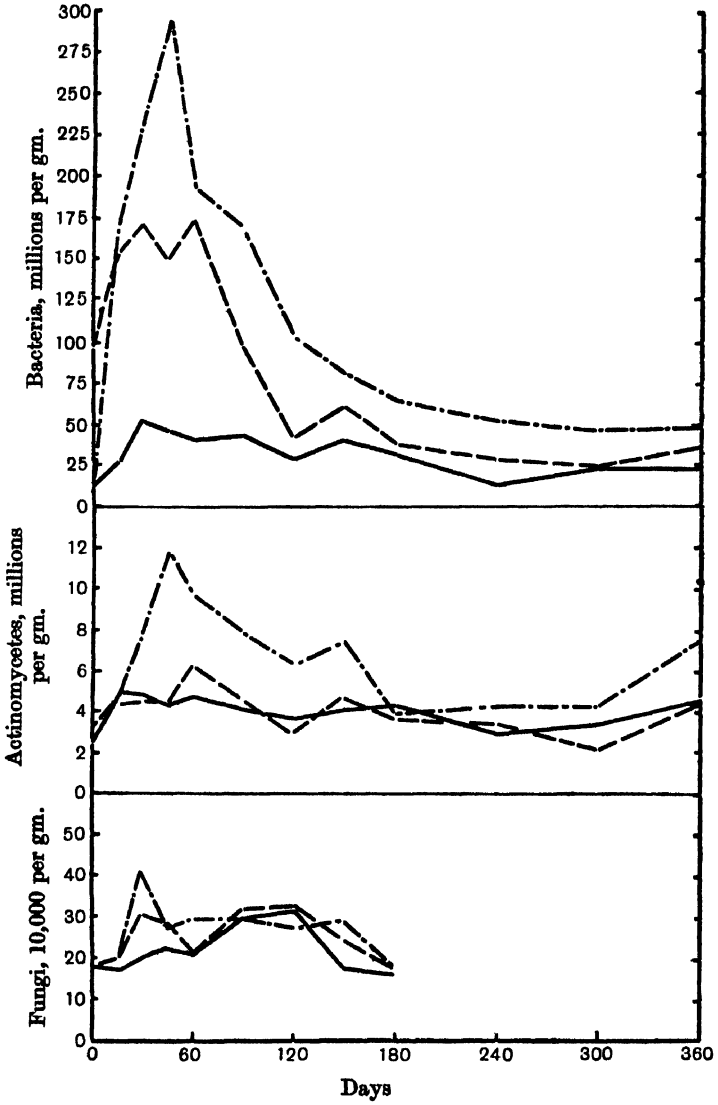

Fig. 5. Numbers of micro-organisms in Hoosfield soil. Third series.

——— Control soil. - - - Soil + fresh manure. —·—·— Soil + Edelmist.

The counts of bacteria, actinomycetes and fungi are shown in Fig. 5. The control soil had quite high numbers of bacteria from 30 to 90 days, after which time the numbers fluctuated on a lower level. The soil +

manure had a high number of bacteria at the start, and this increased greatly during the first 15–60 days, after which time a decrease set in, so that from 120 days the numbers remained practically the same as in the control soil. The Edelmist was poor in living organisms, so that this soil started with a low number of bacteria, but they multiplied rapidly, reaching very high numbers by the 45th day, after which they fell greatly, but still remained in excess over the other two soils. The actinomycetes were but slightly affected by the addition of fresh manure; in the soil with Edelmist they were somewhat stimulated, but not to the same extent as the bacteria. As in the previous experiments, the fungi were not affected to any significant extent.

Table V. *Chemical changes in Hoosfield soil, pH 6·3, with addition of fresh farmyard manure and Edelmist.*

	Control soil		Soil + fresh farmyard manure		Soil + Edelmist	
Time	NO_3-N*	NH_4-N*	NO_3-N	NH_4-N	NO_3-N	NH_4-N
Start	0·6	0·0	0·6	0·2	0·6	6·8
After 30 days	5·0	0·0	3·3	Trace	9·5	1·2
„ 60 „	7·8	0·0	5·6	0·0	10·1	0·0
„ 90 „	8·7	0·0	7·3	0·0	11·6	0·0
„ 120 „	9·2	0·0	9·7	0·0	13·6	0·0
„ 150 „	10·2	0·0	11·1	0·0	18·7	0·0
„ 180 „	10·7	0·0	13·1	0·0	19·5	0·0
„ 240 „	10·1	0·0	14·4	0·0	21·4	0·0
„ 300 „	10·0	0·0	15·4	0·0	23·2	0·0
„ 360 „	12·0	0·0	17·4	0·0	26·5	0·0
Final excess of NO_3 over control, as % of added N	—		13·6		22·9	
α-humus %:						
After 180 days	0·79		1·13		1·38	
„ 360 „	0·64		1·09		1·34	
% N in α-humus:						
After 180 days	3·46		3·66		3·63	
„ 360 „	3·77		3·56		3·62	
Excess of α-humus N over control, as % of added N:						
After 180 days	—		36·5		33·7	
„ 360 „	—		38·1		35·8	
% total N:						
Start	0·165		0·200		0·227	
After 360 days	0·185		0·216		0·239	
% total C:						
Start	1·84		2·58		2·66	
After 180 days	1·70		2·21		2·41	
% added C disappeared:						
After 180 days	—		46·7		28·8	

* mg. per 100 gm. of air-dry soil.

The chemical changes are found in Table V. The control soil showed a gradual accumulation of nitrate, whereas in the soil with fresh manure a marked depression in nitrate formation took place, and lasted for 120 days. The depression was then overcome, and a slow production of nitrate from the manure set in, so that after 300–360 days the excess of nitrate over the control soil corresponded to 13·6 per cent. of the manure N. The Edelmist soil started with a high content of ammonia N, which was nitrified after 30–60 days, but the excess over control was much less than should have been produced by oxidation of the ammonia N added with the manure. There was thus here a relative depression of nitrate formation, which lasted for about 150 days, after which time the amount of nitrate rose, first rapidly, then more slowly, so that at the end of the experiment the excess over control corresponded to 23 per cent. of the manure N. The Edelmist thus showed a nitrifiability much superior to that of ordinary farmyard manure, but the absolute amount of N left untransformed was also larger in the case of Edelmist. It is therefore doubtful whether one is justified in regarding the N compounds of the Edelmist as particularly easily available to the soil micro-organisms—an opinion which has repeatedly been expressed by German investigators, *e.g.* Löhnis (44) and Ruschmann (63). It seems rather that the Edelmist owes its superior value merely to its higher percentage of N, that is, to its more favourable C:N ratio. The humus determinations show that, after 180 and 360 days, the two manured soils had an excess over control in both humus and humus N even larger than that which corresponds to the humus introduced with the manure. These N compounds thus appear to have been perfectly resistant to the attack of micro-organisms for a period of 6–12 months, and a small amount of extra humus seems to have been formed. The N determinations show a rather peculiar phenomenon: N fixation took place in all soils, most in the control and least in the soil with Edelmist.

(5) *Fourth series of experiments.*

In this series a comparison between natural farmyard manure and synthetic farmyard manure made by the "Adco" process (Hutchinson and Richards (28)) was studied.

For this experiment a heavy clay soil, poor in organic matter and of *p*H 6·4, from an unfertilised plot on Hoosfield, was used. It contained an air-dry basis: total N, 0·094 per cent.; total C, 0·0881 per cent.; humus, 0·23 per cent. with 4·59 per cent. N. The natural manure was well fermented and had been well stored. The synthetic manure, prepared from wheat straw and ammonium sulphate, was well decomposed. The composition of the two manures was as follows:

	Natural manure	Synthetic manure
Dry matter (%)	21·5	20·2
Total N, % of dry matter	2·25	2·54
NH_4-N „	0·17	0·03
Total C „	38·2	36·5
Ash „	21·2	29·1
Humus „	17·9	20·7
N in a-humus „	2·30	2·46

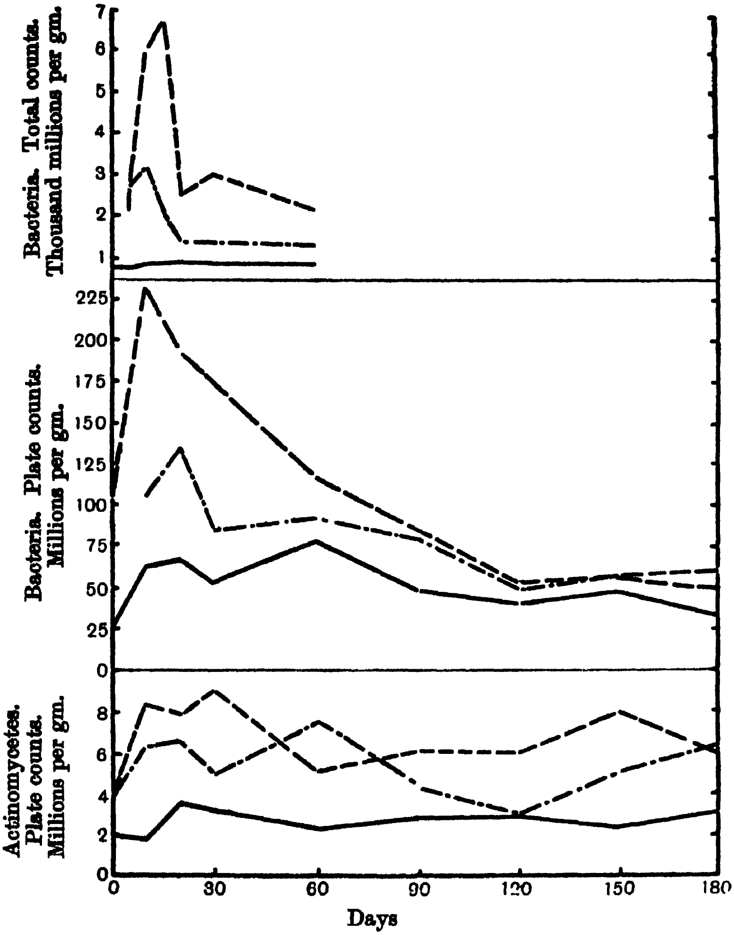

Fig. 6. Numbers of micro-organisms in Hoosfield soil. Fourth series.
——— Control soil. — — — Soil + nat. manure. —·—·— Soil + synthetic manure.

The manures were added in amounts corresponding to 2 per cent. dry matter, and flasks were kept at room temperature for 180 days. In this series bacterial numbers were estimated both by the plate method and by the [illegible] copical method developed by Gray and Thornton (22) in this labo[illegible]

The bact[illegible] counts in Fig. 6 show that the absolute numbers of bacteria foun[illegible] by direct counting were on an average 20 to 30 times as high as those obtained by the plate method, but the initial rise and subsequent fall in bacterial numbers due to addition of manure are reflected in the figures obtained by both methods. The natural farmyard manure caused a very large increase 10–20 days after the addition of the manure. The actinomycetes were also somewhat stimulated by both kinds of manure.

Table VI. *Chemical changes in Hoosfield soil, pH 6·4, with addition of natural and artificial farmyard manure.*

	Control soil		Soil + natural farmyard manure		Soil + synthetic farmyard manure	
Time	NO_3-N*	NH_4-N*	NO_3-N	NH_4-N	NO_3-N	NH_4-N
Start	1·4	0·4	1·4	3·8	1·4	0·9
After 10 days	3·9	0·0	5·7	Trace	6·7	0·0
„ 20 „	4·3	0·0	5·3	0·0	7·8	0·0
„ 30 „	4·5	0·0	5·4	0·0	7·7	0·0
„ 60 „	5·7	0·0	5·3	0·0	14·0	0·0
„ 90 „	4·5	0·0	6·4	0·0	9·8	0·0
„ 120 „	4·6	0·0	6·7	0·0	9·5	0·0
„ 150 „	4·6	0·0	6·6	0·0	—	—
„ 180 „	4·0	0·0	7·0	0·0	9·8	0·0
Excess of NO_3-N over control, as % of added N	—		6·7		11·4	
α-humus %:						
After 180 days	0·23		0·65		0·73	
% N in α-humus	3·91		3·29		3·10	
Excess of α-humus N over control, as % of added N	—		27·8		23·1	
% total N:						
Start	0·094		0·136		0·142	
End	0·094		0·136		0·147	

* mg. of N per 100 gm. of air-dry soil.

The chemical changes are seen in Table VI. The natural manure underwent a slow nitrification in this soil; after 180 days the excess in nitrate N was not even as large as the initial addition of ammonium N. The synthetic manure gave rise at once to a vigorous nitrate formation, so that after 60 days the excess of nitrate corresponded to 16 per cent. of the total N (nearly all organic). After this period the process did not go any further, and there was even a decrease in nitrate content, the cause of which is rather obscure, but which has also been observed by other workers, *e.g.* Glathe (20) and Scheibe (69). The humus determinations again show that the humus of both kinds of manure persisted in the soil, and some more seems to have been formed. The excesses in humus over control soil were 0·42 and 0·50 per cent. for natural and synthetic manure, the quantities added being 0·36 and 0·41 per cent. The amounts of N in humus in the manures amounted to 17·3 and 20·1 per cent. of total N, whereas the excesses in humus N at end of experiment corresponded to 28 and 23 per cent. of the added N. The determinations of total N show that in this case there was no loss of nitrogen.

In these four sets of experiments there was a formation of ammonia and nitrate from the organic N compounds of different kinds of farmyard manure in all cases but two. The first exception was with the strongly acid Park plot soil with manure + straw, in which an abundant fungus flora arose, the second was in the last experiment in which Hoosfield soil with manure was kept for only 180 days. The general results, however, are sufficient to disprove the general validity of the statement of Barthel and Bengtsson (6), that only the ammonium N of the farmyard manure is available for nitrification during the first year. Especially convincing in this respect is the first series of experiments—old farmyard manure in neutral Park plot soil. In this well-decomposed manure, in which practically all N is present in organic compounds, no less than one-fifth of the N has been nitrified after 300 days. In the case of fresh manure it might be argued that the amount of N which underwent nitrification might have been transformed into ammonia, if the manure had been allowed to ferment properly, and the Edelmist may not be entirely comparable with the ordinary farmyard manure. That the C:N ratio of the manure exerts a marked influence on the rate of nitrification can be seen from the following summary:

Series	Material	Time (days)	C:N of added material	% of N nitrified or ammonified
1 A (neutral)	Old manure	500	14·4:1	32
Do.	Do. + straw	500	28·3:1	10
1 B (acid)	Old manure	500	14·4:1	16
Do.	Do. + straw	500	28·3:1	None
2 A (unlimed)	Fresh manure	360	20·6:1	22
Do.	Do. + $(NH_4)_2SO_4$	360	15·9:1	41
Do.	Do. + Do. + straw	360	21·6:1	18
2 B (limed)	Fresh manure	360	20·6:1	21
Do.	Do. + $(NH_4)_2SO_4$	360	15·9:1	27
	Do. + Do. + straw	360	21·6:1	15
3	Fresh manure	360	20·6:1	14
Do.	Edelmist	360	12·8:1	23
4	Natural manure	180	16·9:1	7
Do.	Synthetic manure	180	15·1:1	11

The widening of the C:N ratio means an increased energy supply relatively to the supply of N; this causes the bacteria and other microorganisms to multiply vigorously and to use up more N for the synthesis of their cell substance; not until the death of the cells can this N again be released and transformed into ammonia and nitrate, and in accordance with this we see from Figs. 7–13 *that there is an inverse relationship between bacterial numbers and amounts of ammonium + nitrate N.* In the

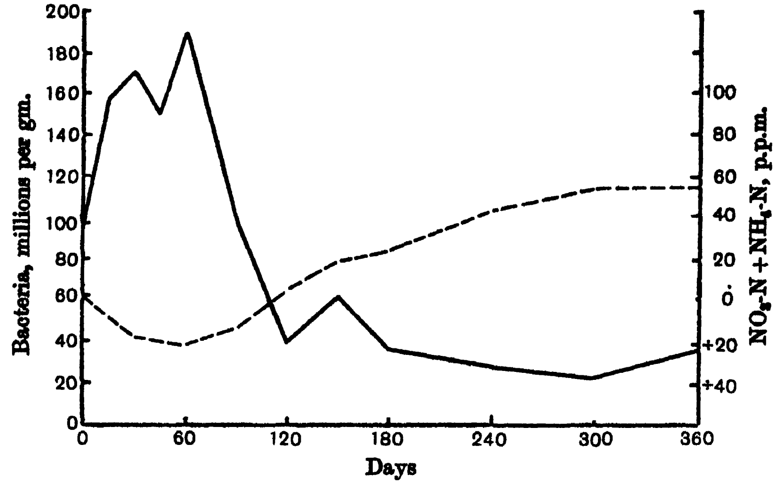

Fig. 7. Bacterial numbers and production of mineral N from farmyard manure in Hoosfield soil.

——— Bacterial numbers. — — — Excess in mineral N over control soil.

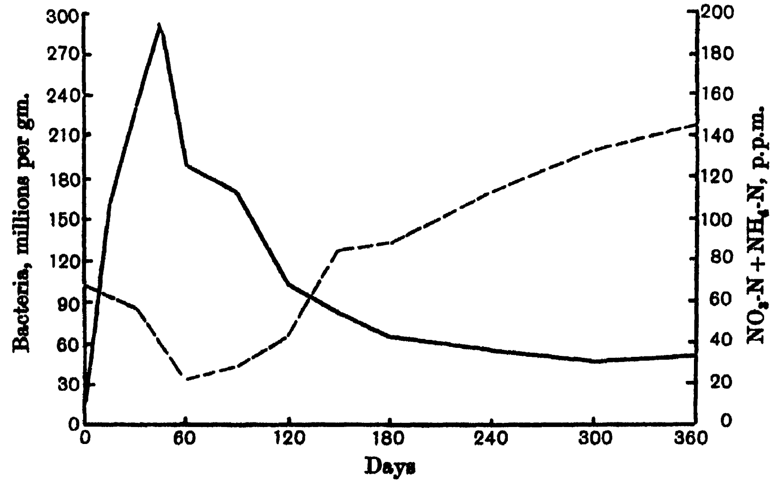

Fig. 8. Bacterial numbers and production of mineral N from Edelmist in Hoosfield soil.

——— Bacterial numbers. — — — Excess in mineral N over control soil.

Woburn soil without straw, where the bacteria do not multiply much, this phenomenon is least marked, and in the acid Park plot soil, where the bacteria are almost inactive, the fungi show a similar relationship.

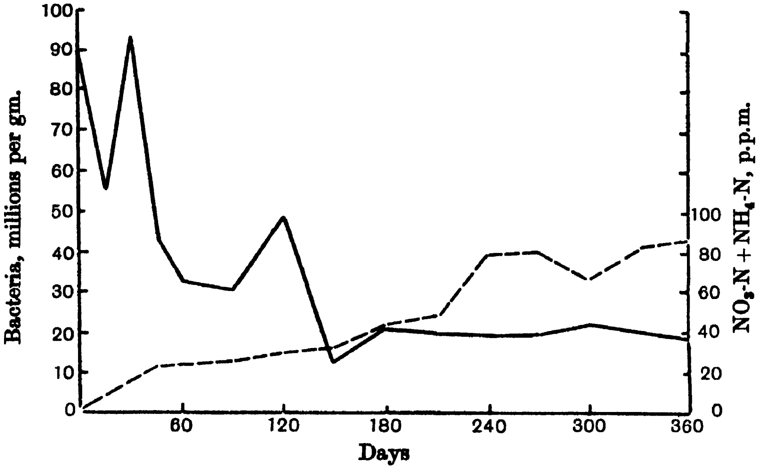

Fig. 9. Bacterial numbers and production of mineral N from farmyard manure in Woburn soil, unlimed.

——— Bacterial numbers. — — — Excess in mineral N over control soil.

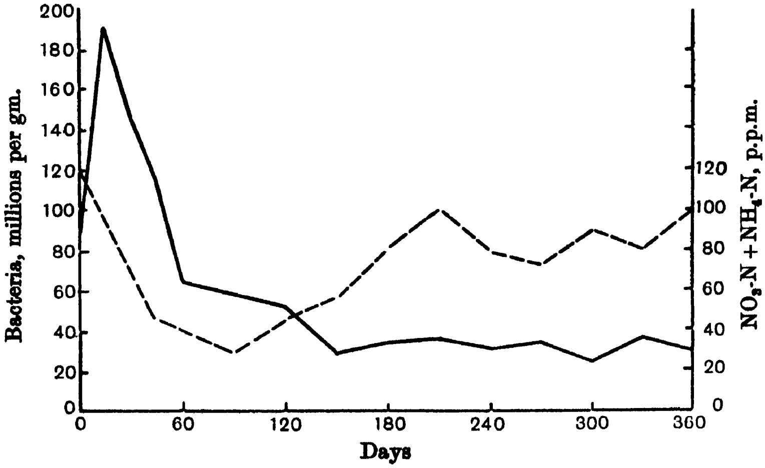

Fig. 10. Bacterial numbers and production of mineral N from manure + $(NH_4)_2SO_4$ + straw in Woburn soil, unlimed.

——— Bacterial numbers. — — — Excess in mineral N over control soil.

If the manure is poor in N (wide C:N ratio) the content of nitrate in the soil is depressed below that of the control soil for a certain period, and even if it is richer in N, a marked formation of nitrate does not appear at once, but begins when the bacterial numbers have again gone down to a low level or some time after this—probably at the time when the dead bacterial cells are in their turn undergoing decomposition. A similar

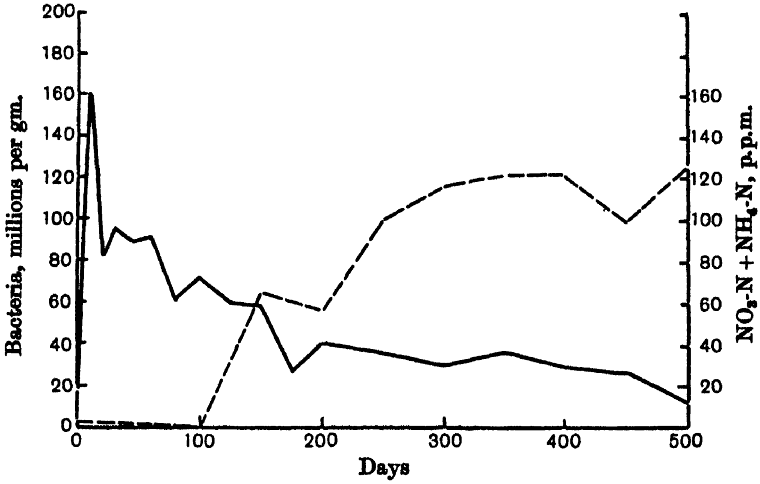

Fig. 11. Bacterial numbers and production of mineral N from farmyard manure in neutral Park plot soil.

——— Bacterial numbers. — — — Excess in mineral N over control soil.

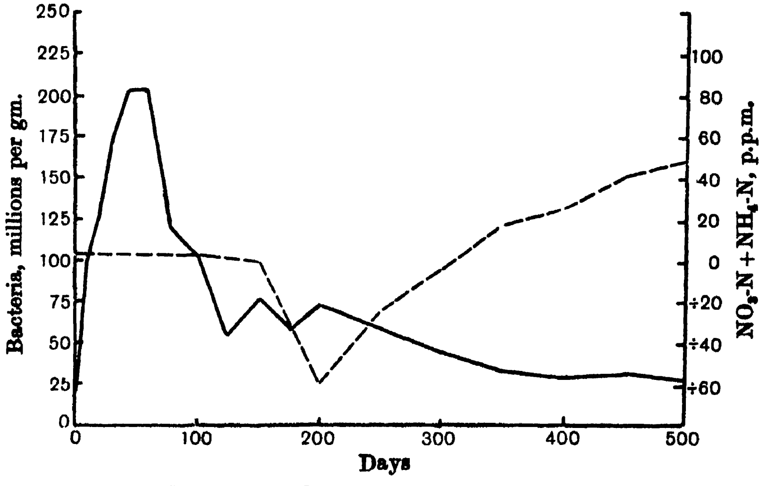

Fig. 12. Bacterial numbers and production of mineral N from farmyard manure + straw in neutral Park plot soil.

——— Bacterial numbers. — — — Excess in mineral N over control soil.

inverse relationship between bacterial numbers and amounts of mineral N can be seen from the data given by Russell and Hutchinson (64), Joshi (31), and Wilson (85).

Although the higher nitrogenous compounds of manure do undergo decomposition, their mineralisation is very slow and incomplete. What is the cause of this? The idea at once suggests itself that, since the excesses in bacterial numbers over control soils continue for a long period, their total numbers might remain so high that the quantity of cell material would account for a considerable part of the manure N locked up at the end of the experiment. A glance at the total counts after 60 days (Fig. 6), however, is sufficient to dispose of this idea.

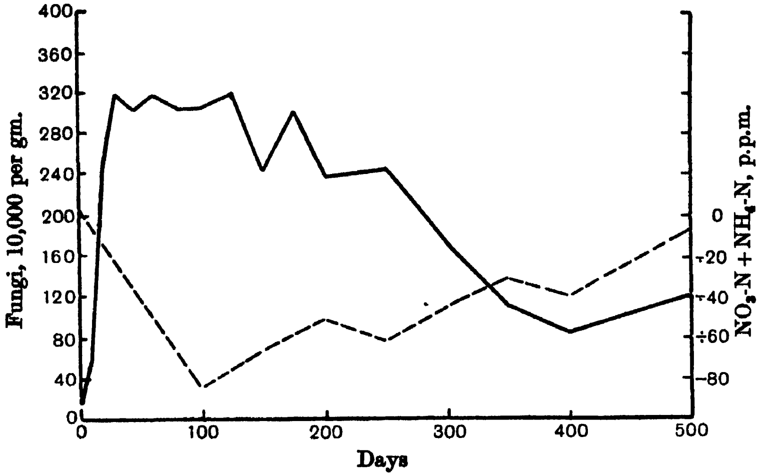

Fig. 13. "Numbers" of fungi and production of mineral N from farmyard manure + straw in acid Park plot soil.
——— "Numbers" of fungi. — — — Excess in mineral N over control soil.

Hoosfield control soil	766	millions of bacteria	per gm.
Soil + natural manure	2204	„	„
Soil + synthetic manure	1309	„	„

A total count in soil from Woburn (second series, unlimed) at the end of the experiment showed in:

Control soil	1560	millions of bacteria	per gm.
Soil + manure	2660	„	„
Soil + Do. + $(NH_4)_2SO_4$	1950	„	„
Soil + Do. + Do. straw	2460	„	„

We have here excesses over control soils corresponding to 400–1500 million cells per gm. of soil. This quantity can be roughly estimated to occupy 40–150 mm. per 100 gm. of soil, assuming that each bacterial cell has a cubic content of 1 μ. If we reckon with a specific gravity of 1 for the bacterial cells, this quantity will be 40–150 mg. per 100 gm. of moist soil, or 50–200 mg. per 100 mg. of air-dry soil. If we assume that the bacterial cells contain 20 per cent. dry matter with 10 per cent. N, we find 1–4 mg. of bacterial N in excess over the control soil. But the quantities of unnitrified manure N at the end of the experiments amount to 30–42 mg., of which that of the bacterial cells thus constitutes only a very small fraction. It is thus evidently not here, in the living bacterial matter, that we have to look for the untransformed and slowly transformable N of the manure. However, the total numbers of bacteria shortly after the addition of manure are very high; for instance, the total counts after 10 days in the Hoosfield soil + natural manure (Fig. 6) show an excess in bacteria over control soil, corresponding to nearly 6000 million cells per gm. of soil; this corresponds per 100 gm. of soil to 100–120 mg. of bacterial dry matter, or 10–12 mg. of N, which is 22 to 25 per cent. of the total amount of N added in the manure. This fact, that a not insignificant part of the manure nitrogen passes into the protoplasm of bacteria, introduces the question of decomposition of dead microbial protoplasm and the possible formation of more or less undecomposable humus-like nitrogenous compounds from this source (Waksman (82)). The N present in the humus fraction of the manure persists in the soil for 180 to 360 days, and sometimes some extra humus is formed. This considerable fraction of the manure N appears to be in a very slightly decomposable state, as the control experiments below also show.

(6) *Decomposition of manures in sand.*

In all the previous decomposition experiments we have relied, as is customary, upon the assumption that the differences in the contents of nitrate, humus, carbon, etc., between a control soil and soil with addition of some organic material, indicate the extent to which the organic material has been attacked. We are, in other words, assuming that the soil's own organic matter is attacked equally in both cases. How far this is true is not, however, known with certainty, and it is not at all unlikely that the addition of organic matter to the soil might protect the soil's organic matter from being attacked. In other cases it seems, as demonstrated by Löhnis (43), that the stimulation of the soil micro-organisms due to addition of organic material involves a greater attack on the soil organic matter itself. To have a control upon this, some decomposition experiments were carried out in sand, a medium which does not in itself contain any matter, from which ammonia or nitrate can be formed, and from which therefore the total production of nitrate becomes an index of the decomposition of the organic material. The following materials were used: (1) Air-dried fresh farmyard manure. (2) Edelmist. (3) Old, badly stored farmyard manure. (4) Humus-free extraction residue of fresh farmyard manure, prepared by treating the manure several times with boiling 4 per cent. NaOH, until a nearly colourless extract was obtained; boiling the residue with 2 per cent. H_2SO_4; washing and drying. The air-dry materials had the following composition:

	Total N (%)	NH_4-N (%)	Total C (%)	Ash (%)
Fresh farmyard manure	1·85	0·01	37·6	25·6
Edelmist	2·86	0·11	42·2	19·3
Old farmyard manure	2·10	0·00	36·6	27·6
Extraction residue	1·23	0·00	37·7	—

The materials were added in quantities of 2 per cent. of dry matter to 300–400 gm. of sand; this mixture was moistened with 12 per cent. of distilled water including 1 c.c. of a suspension of garden soil (1:10) in distilled water. The flasks were kept at 25° C. for 90 days.

Table VII. *Nitrification of various manures in sand.*

	Fresh farmyard manure		Edelmist		Old farmyard manure		Extraction residue of farmyard manure	
Time	NO_3-N*	NH_4-N*	NO_3-N	NH_4-N	NO_3-N	NH_4-N	NO_3-N	NH_4-N
Start	0·0	0·2	0·0	2·2	Trace	0·0	0·0	0·0
After 20 days	4·5	0·6	7·8	0·6	3·1	Trace	0·0	0·0
„ 40 „	5·3	0·0	9·4	0·0	3·4	0·0	0·0	0·0
„ 60 „	6·1	0·0	13·4	0·0	4·2	0·0	0·0	0·0
„ 90 „	7·3	0·0	12·6	0·0	4·1	0·0	Trace	0·0
N added, mg. per 100 gm. of sand	37·0		57·2		42·0		24·6	
C added %	0·752		0·844		0·732		0·754	
C:N ratio, initial	20·3:1		14·8:1		17·4:1		30·6:1	
% of added N nitrified	19·7		22·0		9·8		0·0	
α-humus %: After 90 days	0·33		0·40		0·31		0·28	
% N in α-humus	1·93		2·42		2·16		2·76	
% of added N found in α-humus: After 90 days	16·6		16·8		16·0		—	
Total N, mg. per 100 gm. of sand†: After 90 days	40·2		55·8		41·4		—	
Total C %†: After 90 days	0·426		0·495		0·614		—	
C:N ratio, final‡	12·9:1		11·5:1		14·8:1		—	
% added C disappeared	41·3		39·3		14·1		—	

* mg. per 100 gm. of sand.

† Sand in a control experiment contained 1·6 mg. N and 25 mg. C per 100 gm. This quantity is subtracted here.

‡ NO_3-N subtracted.

The results of the analyses are seen in Table VII. All the manures underwent nitrification, which, as in the soils, proceeded with decreasing rapidity, so that after 3 months 10–22 per cent. of the manure N was nitrified. The Edelmist and fresh manure showed rapid nitrate production, whereas the old manure, in spite of its more narrow C:N ratio, showed a much slower nitrification. The explanation is probably that the C:N ratio of the manure must be reduced to a certain value, usually 10–12:1, before nitrification can start, and this value was reached more slowly in the case of old manure, which consisted chiefly of old resistant residues.

The humus determinations showed a little decrease, though perhaps not significant, in humus N in fresh manure and in old manure, and, especially in Edelmist, the decomposition of this fraction appeared to be definite, though not considerable. The total N determinations show that none of the sand-manure mixtures lost significant quantities of N.

The manure residue with its wide C:N ratio did not undergo any nitrification, but the humus determination shows the interesting fact that a considerable amount of humus, containing 2·8 per cent. N, was formed from this material, which at the start did not contain any alkali-soluble material. It would probably be premature to claim that this humus was synthesised by micro-organisms active in the sand. The lignins are only partly soluble in alkali, and it is possible that the humus found here was mostly derived from lignin which, during the incubation, perhaps under the influence of micro-organisms, became soluble in alkali.

(7) *Decomposition of humus.*

Finally, an experiment was carried out in order to compare the decomposition of humus from manure and from soil. The manure humus was prepared from air-dry manure and Edelmist by extraction with boiling 4 per cent. NaOH, precipitation of the filtrate with H_2SO_4, boiling the precipitate in excess of H_2O (about 2 per cent. solution), filtering, washing till free from acid, and drying. The soil humus was α-humus from a fertile, loamy garden soil. The composition of the materials was:

	Total N (%)	Total C (%)	Methoxyl (%)	Ash (%)	Moisture (%)
Fresh manure humus	3·11	55·7	5·65	0·4	6·3
Edelmist humus	3·66	59·0	5·88	1·2	6·1
Soil humus	3·97	52·2	3·16	1·3	6·2

Two per cent. of the two sorts of manure humus and 1 per cent. of soil humus were added to 150 gm. portions of sand which further received

0·5 per cent. $CaCO_3$ and 12 per cent. of a 0·1 per cent. solution of K_2HPO_4 + 1 c.c. of a suspension of garden soil. The mixture was kept in round flasks of about 300 c.c. capacity for 240 days at 25° C.

Table VIII. *Decomposition of humus from manure, Edelmist and soil in sand culture.*

	Manure humus (2 %)		Edelmist humus (2 %)		Soil humus (1 %)	
Time	NO_3-N*	NH_4-N*	NO_3-N	NH_4-N	NO_3-N	NH_4-N
Start	0·0	0·0	0·0	0·0	0·0	0·0
After 60 days	Trace	Trace	0·0	0·0	0·0	Trace
„ 120 „	0·0	0·0	0·0	0·0	2·7	0·0
„ 180 „	0·0	0·0	2·3	0·0	4·7	0·0
„ 240 „	0·0	Trace	2·5	Trace	5·0	0·0
% of N nitrified	None		3·4		12·6	
% C at start	1·11		1·18		0·52	
% C at end	0·86		1·07		0·44	
C:N initially	17·9:1		14·8:1		13·1:1	
C:N finally (min. N subtracted)	13·8:1		15·2:1		12·7:1	

* mg. per 100 gm. of sand.

Table VIII shows that the soil humus underwent a slow, but definite, nitrification; about 12 per cent. of its N was nitrified after 240 days, in which time 15 per cent. of its C also disappeared, so that its C:N ratio remained nearly the same. The Edelmist humus was more resistant; not until after 7–8 months was there a definite, although slight nitrification. The manure humus with the wider C:N ratio was still more slowly nitrified, but its carbon compounds were attacked to a larger extent, so that this material tended to adjust itself to the same C:N ratio as the others. Microscopic examinations showed the presence of a large number of small rods and cocci, especially in the sand + manure humus. We have only a few records in the literature, of laboratory experiments on decomposition of humus. Rimbach (62) added Ca-humate with 4 per cent. N to sand and found 5·9 per cent. of its N nitrified after 2 months. Nikitinsky (61) found humic acid decomposed both in the presence and absence of living micro-organisms, but more rapidly in the former case. Fraps (18) added humic acid to soil and found about 3 per cent. of its C given off as CO_2 in 6 weeks under conditions where cotton-seed meal and manure lost 62 and 30 per cent. of their C. Löhnis and Green (41) found that humus from peat was very slowly nitrified in the soil, humus from manure more rapidly, and humus from plant materials (probably containing some easily decomposable protein material) still more rapidly;

the rate of nitrification was, to a certain degree, proportional to the percentage of N. Liesche (38) prepared humus from Edelmist and found that 2·5–3·4 per cent. of its N was nitrified and 1·5–2·1 per cent. of its C disappeared in soil or sand after 4 weeks. The material was not boiled in acid, so that some easily decomposable N compounds may have been present, which may account for the discrepancy with the present results. The only observation of actual decomposition of humus by bacteria is due to Winogradsky (86), who found colonies of small rod-shaped bacteria developing on silica gel plates with Ca-humate. The colonies very slowly formed transparent haloes.

All these observations show conclusively that the nitrogen in the humus fraction of the manure is present in a state where it is very slowly mobilised by the soil micro-organisms, a fact which was suspected almost 60 years ago by Dehérain (11), whose words can still be fully endorsed to-day: "Quant à la matière noire, dernier terme de la fermentation du fumier, il est probable qu'elle résiste plus longtemps, que ce n'est que lentement qu'elle se brûle, mais qu'elle fournit encore des nitrates comme dernier produit d'oxydation. Elle constitue sans doute la plus grande partie de cette *vieille force*, qui s'accumule dans les terres bien fumées."

(8) *Origin of the humus fraction.*

The question now arises: what is the origin of this humus fraction? It has sometimes been suggested that the process of condensation between sugars and amino acids discovered by Maillard (45) might be responsible for the formation of humic matter both in soil and manure. Against this Waksman (80) and Liesche (38) have pointed out that sugars and amino acids are never found in the soil except in mere traces and, moreover, du Toit (74) and Marshall (46) found that the product of this reaction has physico-chemical properties which are quite different from those of soil humic acid. In manure it is conceivable that this process might take place, especially in Edelmist, where the temperature for a long time remains at 50–60° C. Löhnis (44) even speaks of this process as *the* mode of formation of humus in Edelmist. The weak point of the theory is that the process seems to have been studied only in concentrated solutions of sugars and amino acids. A small experiment on the importance of the concentration was carried out.

1. 50 c.c. of a sterile solution of 2 per cent. dextrose and 0·5 per cent. glycine (solutions sterilised apart and mixed) were kept in a cotton-plugged flask at 54° C. After 5 days the fluid had a slight yellow tinge; after 10 days it was straw coloured; and after 21 days, when its original

volume through evaporation had been reduced to 10 c.c., it was deep orange. No precipitate was obtained on acidification with hydrochloric acid.

2. 50 c.c. of a sterile solution of 4 per cent. dextrose and 1 per cent. glycine kept at 54° C. After 2 days a faint yellow tinge appeared and became gradually more intense, until after 24 days the solution was coffee-brown. No precipitate on acidification. The solution had evaporated down to 10 c.c.

3. Same solution kept at 37° C.: straw yellow after 24 days.

4. 50 c.c. of a sterile solution of 8 per cent. dextrose and 2 per cent. glycine at 54° C. Solution became orange after 5 days, and after 14 days, when it had evaporated down to 15 c.c., it was intensely brown, and transparent when diluted. On acidification with HCl it yielded 0·050 gm. of an amorphous, dark brown precipitate containing 8 per cent. N.

This simple experiment shows that there is indeed a formation of coloured matter, but even in solution of 4 per cent. dextrose and 1 per cent. glycine no production of any substance precipitated by acid, which might be accepted as α-humus or humic acid, after more than 3 weeks' incubation at 54° C. (which is about the temperature of Edelmist after the first days of aerobic fermentation), even when we allow for the increase in concentration due to evaporation. And such a concentration of sugars and amino acids is hardly imaginable in a medium where living organisms are constantly present (the Edelmist is indeed poor in bacteria, but never sterile, and thermophilous organisms would probably flourish to an enormous extent if such concentrations of excellent nutrients like sugars and amino acids arose). It is thus evidently not here that we have to look for the source of nitrogenous humus. But the studies of recent years have indicated that lignins are the source of the bulk of the soil humus, although the fraction containing N must have another origin. Straw, which is present in abundance in the manure, contains about 20 per cent. of lignin, which thus is most likely to be found in the humus of the manure. The determinations of methoxyl in the humus as well as the carbon determinations also point definitely in this direction. But what is the source of the comparatively high N content of the humus? It was suggested in 1888 by Dehérain (12) that the humus of manure may be a mixture of lignins and proteins built up by bacteria, and it is most likely that some protein material will be extracted and precipitated along with the lignin. We cannot, however, be dealing with an ordinary protein, mechanically mixed with the lignin, since normal proteins are easily attacked by bacteria (Balks (4)), and in such a case we should have expected that the decomposition experiment with humus would have shown at first a rapid ammonia or nitrate production, later perhaps followed by a consumption of these compounds, when the lignins begin to undergo decomposition. Hobson (25) suggested that soil humic acid may be regarded as an adsorption compound between lignins and certain proteins, which latter are protected through the adsorption against bacterial attack, and he demonstrated that a composite of lignin and albumen, obtained by mixing alkali solutions of the two compounds and precipitating them together by means of acid, shows an amino N distribution almost identical to that of soil humic acid, when analysed by the van Slyke method. Some experiments were carried out in order to test this point. Mixtures of lignin and protein were prepared by mixing NaOH solutions of carbohydrate-free lignin[1] and casein and precipitating the mixture by means of HCl. Three samples containing 3·9–4·1 per cent. N were prepared, and, when added to mineral nutrient solution inoculated with soil suspension and kept at 25° C., only a trace of ammonia formation took place after 3 weeks, whereas in a control solution containing a corresponding amount of N as casein, 75–80 per cent. of this was ammonified. However, in a similar experiment carried out in soil, this resistance to microbial attack was not nearly so marked, and only an insignificant part of the added N was recovered in the humus after 45 days. It does not seem that lignin will form such resistant adsorption compounds with every protein, but the suggestion is interesting and should be tested further. A third possible source of nitrogenous humus is the microbial protoplasm, which may contain compounds that are in themselves very resistant or perhaps able to form resistant compounds with the lignin.

DISCUSSION.

All these experiments show us that the N of farmyard manure is nitrified rather slowly and never completely—a result which is in agreement with our experience from field experiments. The degree of nitrification does not depend so much on whether the N is present as ammonia or organic compounds, as on the C:N ratio of the manure and the content of relatively undecomposable humus-like compounds. When added to the soil, the manure introduces a large quantity of energy material, which enables the micro-organisms of the soil to develop abundantly for a shorter or longer period, especially when fresh straw is present in the manure. This multiplication of micro-organisms entails the locking up of considerable amounts of N in protoplasm, and the nitrification is therefore retarded, until the numbers of micro-organisms have again decreased. The rise in nitrate production, which sets in at this period, corresponds apparently to the decomposition of the dead bacterial bodies. The nitrification does not become complete within 16 months; but its rate gradually diminishes, and a considerable residue of nitrogenous compounds resists decomposition. A part of this is evidently the humus fraction of the manure, but this does not account for all of it. An explanation for the origin of these residues will be sought in a following section of this work.

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[1] Kindly supplied by Mr G. V. Jacks, B.A., B.Sc., Chemical Department.

The remarkable losses of N in the manured soils are difficult to account for. The only microbial process which is known with certainty to give rise to a development of elementary N is denitrification. That this may have occurred in the Woburn soil is possible, though not certain, and it is unlikely that this should be responsible for the heavy losses of N repeatedly observed in manured, tilled soil. These losses seem connected with good aeration, which is not favourable to denitrification (although not at all detrimental to the existence of the denitrifying bacteria themselves). In accordance herewith, Russell and Richards (65) found no loss of N from manure decomposing under perfectly aerobic or perfectly anaerobic conditions, whereas a considerable loss occurred under semi-aerobic conditions. These latter would be more likely to exist in an untilled than in a tilled soil, from which the losses of N are heavy (Shutt (72)). Apart from denitrification, the possibilities of losses of elementary N through microbial processes are only few and little known. The *B. azotofluorescens*, claimed by Kaserer (32) to be able to oxidise ammonia to water and free N, has not been found by other investigators. Schittenhelm and Schröter's (70) observation that N is evolved from nucleic acid when decomposed by *B. coli*, was shown by Oppenheimer (55) to depend on analytical errors. Pfeiffer and Lemmerman (57) found that soil with addition of manure and nitrate lost N to an extent which could hardly be ascribed to denitrification alone, and Sabachnikoff (67) came to a similar conclusion. Recently Lemoigne and Dopter (37) claim to have isolated a number of bacteria and actinomycetes, which cause a loss of considerable amounts of N from pure cultures under conditions where precautions are taken against the evaporation of ammonia. Their very brief paper unfortunately does not give any idea either of the identity of the organisms or the biochemical nature of the processes which cause the losses of N.

There is thus a certain discrepancy between the field and the laboratory experiment. In the former case losses of N seem to be the rule, but in the experiments described here there was a significant loss of N only in the limed Woburn soil, although the additions of manure were in all cases enormous. The experiments in Hoosfield soil and pure sand show that the decomposition of manure can go very far without a significant loss of N occurring, as was also found by Joshi (31). The possibility remains that we are here neglecting a physico-chemical factor, namely the influence of light, which is of course far stronger in the field than in the laboratory experiments. Berthelot (7) observed that humic acid, when exposed to light, absorbs O_2 and produces CO_2; this observation was later definitely confirmed by Nikitinsky (51). It seems not impossible that such a purely chemical decomposition of humus might give rise to an evolution of free N.

SUMMARY.

Decomposition experiments were carried out in the laboratory with different kinds of farmyard manure in various soils (sand and clay, acid and neutral). In neutral or slightly acid soil there was a very strong multiplication of bacteria and, to a smaller extent, of actinomycetes immediately after the addition of manure. This increase, which was especially marked when fresh straw was present in the manure, was sooner or later followed by a rather sudden decrease, which caused the numbers of bacteria gradually to approach those in the control soils. The actinomycetes were generally more abundant in the later stages of the process. This suggests that they may be especially active in the decomposition of the more resistant residues. The "numbers" of fungi were not affected by the addition of manure alone (except to a slight degree in strongly acid soil, where the bacteria seemed inactive), but the presence of fresh straw caused them to become active, especially in strongly acid soil, where their "numbers" remained at a very high level for a long time, this abundance of fungi consisting of both mycelium and spores.

The nitrification of the manure N became active at the period when the bacterial, or fungal, numbers were again decreasing. The statement that the organic N of the manure does not undergo any nitrification during the first year could not be confirmed, but the nitrification of the organic N was found to be incomplete, since it became gradually slower and seemed to tend to come to a standstill. The α-humus fraction of the manure contained a considerable amount of N in a very inert form.

This fraction, which contained 18–25 per cent. of the total organic N of the manure, did not undergo any significant decomposition in the soil during 6–12 months, after which time it could be recovered in the α-humus fraction of the soil, often together with some extra humus, which seemed to have been synthesised during the process of decomposition. The humus fraction of the manure consisted largely of lignin, probably combined with some proteid material.

The C:N ratio of the manure exerted a great influence upon the degree and the rapidity of the nitrification of the manure. The so-called Edelmist showed a stronger nitrification than ordinary farmyard manure, but this seemed merely dependent on its narrower C:N ratio; its N compounds did not seem more easily decomposable than those of ordinary manure.

Control experiments with dried manure in sand gave similar results, but the decomposition seemed to proceed somewhat more rapidly than in the soils. The manures tended to adjust themselves to a C:N ratio of 11–12:1, and no loss of N took place.

In the soils there was in some instances a significant loss of total N, which might be due to denitrification.

The comparatively low fertilising value of the organic N of the manure seems to depend on the following phenomena: the organic matter of the farmyard manure is a mixture of compounds of a fairly wide C:N ratio. When this is added to the soil, the various compounds are attacked by the bacteria, actinomycetes and fungi, and a part of the available N of the manure is used up as nitrogenous food by the micro-organisms. When the supply of readily available energy material is exhausted, the bacterial numbers drop, and a production of mineral N begins. This production diminishes gradually without, in any case, reaching the total amount of N in the manure. In this respect the farmyard manure resembles other organic manures, which generally yield only a fraction of their N as nitrate, but here the phenomena are somewhat more complicated owing to the presence of the resistant humus fraction in the manure.

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THE MICROBIOLOGY OF FARMYARD MANURE DECOMPOSITION IN SOIL.

II. DECOMPOSITION OF CELLULOSE.

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In a previous paper (12) it was pointed out that the marked increase in bacterial numbers immediately after the addition of manure to the soil is inversely correlated with the production of ammonia and nitrate from the manure, apparently because the bacteria derive the energy for their increase mainly from non-nitrogenous compounds, and assimilate the available nitrogen which is not released again until after the death and subsequent decomposition of the bacterial cells. Cellulose and lignins represent the main groups of energy material in well decomposed manure while fresh manure and straw also contain pentosans in considerable amount. It is presumably from the cellulose and pentosans that the organisms derive the energy for their initial increase, since numerous studies have agreed in showing that the lignins are only very slowly decomposed and contribute largely to the formation of humus in soil and manure. The present contribution deals with the decomposition of the cellulose.

The micro-organisms capable of decomposing celluloses are of widely different groups:

1. *Aerobic mesophilic bacteria* (v. Iterson (11), McBeth and Scales (18), Hutchinson and Clayton (10), Gray and Chalmers (8), Winogradsky (32), Dubos ( ), Kalninš (13)).
2. *Anaerobic mesophilic bacteria* (Omeliansky (19), Khouvine (14)).
3. *Thermophilic bacteria* (Pringsheim (21), Kroulik (16)).
4. *Actinomycetes* (Krainsky (15)).
5. *Filamentous fungi* (v. Iterson (11), Daszewska (3), McBeth and Scales (18), Otto (20), Waksman and Heukelekian (9, 28), Rege (22)).
6. *Higher fungi* (Tubeuf (26), Malenkovic (17), Wehmer (31), Rege (22)).

Of these groups the anaerobic bacteria are hardly active in normal soils (Waksman and Skinner (29), Dubos (4)), although undoubtedly of importance in the manure heap. The same is probably true of the thermophilic bacteria. The actinomycetes mostly exert only a slight influence upon the cellulose and seem of little importance except in dry soils (Dubos (4)). The higher fungi are extremely important agencies in decaying wood (see Thaysen and Bunker (24)) and consequently in forest soil, but their importance in field soil is unknown, although it may be considerable. We are thus left with the groups 1 and 5, viz. the aerobic mesophilic bacteria and the filamentous fungi, which both become active when cellulosic material is added to the soil (Waksman and Heukelekian (28), Waksman and Skinner (29), Dubos (4), Winogradsky (32)). The importance of these organisms in the decomposition of farmyard manure in the soil has not received much attention, and most of the work has been carried out with pure cellulose, a compound occurring in nature in far smaller amounts than the lignified cellulose of straw and wood, which is much more slowly decomposed (Barthel and Bengtsson (1), Rege (22)). In the present contribution the following questions have been considered:

1. What types of cellulose-decomposing micro-organisms are active, when farmyard manure is decomposed in the soil?
2. How large amounts of nitrogen are assimilated by the various organisms in proportion to the amounts of decomposed cellulose?
3. Are humus-like nitrogenous compounds formed by the cellulose-decomposing micro-organisms?

I. TYPES OF CELLULOSE-DECOMPOSING ORGANISMS IN VARIOUS SOILS WITH ADDITION OF MANURE AND STRAW.

In three of the series of manure decomposition experiments described in the previous paper (12) determinations of the relative abundance of cellulose-decomposing bacteria were carried out by means of the dilution method used by Dubos (4): test-tubes with strips of filter-paper (Whatman No. 41) half immersed in a mineral nutrient solution[1] were inoculated with 1 c.c. portions of soil suspension of increasing dilution, and incubated at 25° C. for 3–4 weeks. In cases where stimulation of the soil fungi (determined by the plate method) had taken place, the more conspicuous forms were isolated and tested for their ability to grow on cellulose in the form of filter-paper.

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[1] $NaNO_3$, 2·0 gm.; K_2HPO_4, 0·5 gm.; $MgSO_4$, 0·2 gm.; NaCl, 0·2 gm.; $FeCl_3$, trace; distilled water, 1000 c.c.

The following results were obtained:

SERIES 1A.

Neutral Park plot soil, pH 7·0, *with addition of manure and oat straw.*

(*a*) Results of the dilution method; tubes incubated 100 days at room temperature:

Control soil. Four tubes inoculated with soil suspension diluted 1:200 showed the paper in two tubes broken at level of solution and in two not attacked.

Soil + manure. Four tubes inoculated with soil suspension diluted 1:1000 showed the paper in all tubes broken at level of solution, which was colourless to pale yellow. Four tubes inoculated with soil suspension diluted 1:10,000 showed the paper in three tubes broken, and in one not attacked.

Soil + manure + straw. Four tubes inoculated with soil suspensions diluted 1:10,000 showed the paper in three tubes broken at level of solution, and in one yellow zone. Five tubes inoculated with soil suspension diluted 1:100,000 showed the paper in three tubes broken at level of solution, and in two not attacked.

The microscopic picture of the paper from the tubes showing an attack was the same in all cases: an abundance of small, actively motile, *Vibrio*-like organisms were present. Pure cultures of these could be obtained by streaking the liquid on an agar medium with mineral nutrients and finely divided hydrocellulose prepared by the method of Scales (23). When incubated for 6–8 days at 25° C., small colourless colonies appeared along the streaks, surrounded by very distinct clear haloes. When transfers were made to filter-paper, decomposition of this would set in after a few days' incubation at 25° C. One strain, isolated from soil + manure and termed *Vibrio* B.M., was kept for further study. Young cultures (2 days) on filter-paper: slightly curved, slender rods, 1·7–2·5 × 0·5–0·6μ, actively motile by means of one polar flagellum. In older cultures short, almost coccoid, forms are seen. Gram-negative. Endospores are not formed. Nitrate is not reduced. Starch is hydrolysed. Agar is not liquefied. Strictly aerobic. Growth is better at 25° C. than at 20° C.; no growth at 37° C. On filter-paper strip in mineral nutrient solution the attack is already visible after 2 days, and after 3 days the paper breaks into two at the level of solution, where a narrow zone of the paper is transformed into a soft pulp. The solution becomes faintly turbid, and a faint lemon-yellow pigment is formed in the paper. The mode of attack is much the same as that of *Vibrio* (*Microspira*) *agarliquefaciens* (Gray and Chalmers (8)), to which the organism appears closely related.

(*b*) *Activity of fungi.* As shown in the previous paper, the manure caused no increase of fungi in this soil, but the addition of straw gave rise to an enormous development of fungi, particularly of *Cephalosporium* sp., which, however, did not decompose cellulose in pure culture. Special fungi, to which the plate method is not readily applicable, seemed, however, to be active here. A plating on cellulose agar from soil + manure yielded a peculiar fungus—apparently a *Botryosporium*, forming numerous clusters of black chlamydospores which decomposed cellulose very actively in pure culture. The same fungus could be obtained by planting the half-decomposed bits of straw from the soil + manure + straw directly into plates of cellulose agar. The fungus appeared remarkably sensitive to acid reaction; this was probably the reason why it was not obtained by the plate method.

SERIES 1B.

Acid Park plot soil, pH 3·8, *with addition of manure and straw.*

(*a*) Results of the dilution method; tubes incubated for 100 days at room temperature:

Control soil. Two tubes inoculated with soil suspension diluted 1:100; one tube showed a doubtful attack and one a development of fungi.

Soil + manure. Two tubes inoculated with soil suspension diluted 1:100 showed in one tube a doubtful attack, and in one a development of *Spirochaeta cytophaga.* Two tubes inoculated with soil suspension diluted 1:5000 both remained sterile.

Soil + manure + straw. Two tubes inoculated with soil suspension diluted 1:200 showed in one tube a development of fungi and in one of *Spirochaeta cytophaga.* Two tubes inoculated with soil suspension diluted 1:5000 showed one tube with a development of fungi and one sterile.

There is thus no significant development of cellulose-decomposing bacteria in this very acid soil. The presence of *Spirochaeta cytophaga,* which occasionally develops in low dilutions, may be due to its having been introduced with the manure.

(*b*) *Activity of fungi.* As shown before, the soil with manure and straw showed an enormous development of fungi, especially *Trichoderma* sp. and *Amblyosporium* sp. (?), forms which both grew well on cellulose in pure culture.

SERIES 2 A.

Faintly acid sandy soil from Woburn (*p*H 5·5–5·8), *with addition of manure, ammonium sulphate, and straw.*

(*a*) Results of the dilution method after 45 days' incubation at room temperature:

Soil	Dilution	Tube no.	Development of micro-organisms
Control	1:200	*a*	Sterile
		b	
		c	
Soil + manure	1:250	*a*	Paper broken, colourless
		b	"
		c	Sterile
	1:2500	*a*	Paper broken, colourless
		b	"
		c	Yellow spots, paper not attacked
Soil + manure + $(NH_4)_2SO_4$	1:250	*a*	*Spirochaeta cytophaga*
		b	
		c	
	1:2500	*a*	Sterile
		b	
		c	
Soil + manure + $(NH_4)_2SO_4$ + straw	1:5000	*a*	*Spirochaeta cytophaga*
		b	
		c	

Repeated examination after 90 days:

Control, *p*H 5·7	1:200	*a*	Paper broken, colourless
		b	Fungi
		c	"
Soil + manure, *p*H 5·8	1:25,000	*a*	Paper broken, colourless
		b	
		c	
		d	
Soil + manure + $(NH_4)_2SO_4$, *p*H 5·5	1:2500	*a*	Fungi
		b	"
		c	Sterile
		d	"
Soil + manure + $(NH_4)_2SO_4$ + straw, *p*H 5·7	1:2500	*a*	Fungi
		b	"
		c	Sterile
		d	"

Spirochaeta cytophaga was active to some extent in the manured soils during the first 45 days, especially in the soil with manure and straw, and organisms of the *Vibrio*-type were active in the least acid soil (soil + manure), where they continued to flourish after 90 days. In the soils of *p*H 5·5–5·7 no activity of cellulose-decomposing bacteria was apparent after 90 days.

(*b*) *Activity of fungi.* The plate counts showed a considerable development of fungi in soil + manure + ammonium sulphate + straw, especially of a *Monosporium* sp., which made a good growth on filter-paper in pure culture.

SERIES 2B.

Same soils and additions as Series 2A, +1 per cent. $CaCO_3$, *pH* 6·5–6·7.

(*a*) Results of the dilution method after 45 days:

Soil	Dilution	Tube no.	Development of micro-organisms
Control	1:200	*a*	*Spirochaeta cytophaga*
		b	"
		c	Fungi
	1:2000	*a*	*Spirochaeta cytophaga*
		b	Sterile
		c	"
Soil + manure	1:25,000	*a* *b* *c*	Paper broken, colourless
	1:100,000	*a*	Paper broken, colourless
		b	"
		c	Sterile
Soil + manure + $(NH_4)_2SO_4$	1:25,000	*a* *b* *c*	Sterile
Soil + manure + $(NH_4)_2SO_4$ + straw	1:25,000	*a* *b* *c*	Paper broken, colourless
	1:100,000	*a*	Paper broken, colourless
		b	"
		c	"
		d	Sterile

Examination after 90 days gave similar results.

There was thus an abundant development of bacteria of the *Vibrio*-type in these nearly neutral soils, except in the soil with ammonium sulphate. Microscopically the cultures appeared like those of Series A1. A strain termed *Vibrio* W.6 was isolated from soil + manure. It appeared much like *Vibrio* B.M., but did not form any yellow pigment and reduced nitrate to nitrite.

(*b*) *Activity of fungi.* The plate counts did not show any significant stimulation of the fungi in these soils.

SERIES 3.

Faintly acid clay from Hoosfield (pH 6·3), with addition of farmyard manure and Edelmist.

(*a*) Results of the dilution method after 30 days at room temperature:

Soil	Dilution	Tube no.	Development of micro-organisms
Control	1:200	*a*	*Spirochaeta cytophaga*
		b	„
		c	Paper broken, colourless
		d	Sterile
Soil + farmyard manure	1:5000	*a*	Paper broken, colourless
		b	
		c	
	1:25,000	*a*	Paper broken, colourless
		b	
		c	
		d	
Soil + Edelmist	1:5000	*a*	*Spirochaeta cytophaga*
		b	
		c	
	1:25,000	*a*	Paper broken, colourless
		b	*Spirochaeta cytophaga*
		c	Sterile
		d	„

There is here again a decided stimulation of the *Vibrio*-like organisms in soil + ordinary manure, and of *Spirochaeta cytophaga* in soil + Edelmist. An organism, termed *Vibrio* H.M., was isolated from soil + manure. It resembled *Vibrio* B.M., but it did not form any yellow pigment, and its cells were somewhat thinner (0·3–0·4 μ).

(*b*) *Activity of fungi.* No significant stimulation occurred in any of the soils.

All these experiments show a definite stimulation of cellulose-decomposing bacteria due to addition of manure and straw in faintly acid to neutral soils (*p*H 6·3–7·0). At *p*H 5·7–5·8 the multiplication of these organisms is far less pronounced, and at *p*H 5·5 it seems quite absent. *Spirochaeta cytophaga* has a tendency to develop at the faintly acid reactions (*p*H 5·8–6·3), whereas the vibrios are decidedly more abundant at reactions nearer to the point of neutrality.

In order to see whether similar results can be obtained with other soils, some control experiments were carried out by adding cellulose as filter-paper or ground wheat straw to soils of different reaction. This also gives us a control on the source of errors possibly due to the introduction of large numbers of living cellulose-decomposing bacteria in fresh farmyard manure.

First control experiment.

Soils of *p*H values from 4·3 to 7·1 received 1 per cent. filter-paper or wheat straw, distilled water to approximately 70 per cent. of the water-holding capacity, and 21·3 mg. of N as ammonium phosphate per 100 gm. of dry soil. Samples of 200 gm. were kept in a moist condition at room temperature.

The following soils were used:

(1)	Clay soil from Cheshire	*p*H 4·3
(2)	Sandy soil from Tunstall	*p*H 4·9
(3)	Mixture of soils 1 and 4	*p*H 5·9
(4)	Clay soil from Hoosfield	*p*H 6·2
(5)	Garden soil, heavy loam	*p*H 7·1

The dilution method gave the following results:

1 (*a*). *Cheshire soil pH* 4·3 + *filter-paper.* No development of cellulose decomposing bacteria after 20 or 45 days.

1 (*b*). *Do.* + *straw.* No cellulose decomposing bacteria after 20 or 45 days.

2 (*a*). *Tunstall soil, pH* 4·9 + *filter-paper.* No cellulose decomposing bacteria after 20 or 45 days.

2 (*b*). *Do.* + *straw.* No cellulose decomposing bacteria after 20 or 45 days.

3 (*a*). *Mixed soil, pH* 5·9 + *filter-paper.* No cellulose decomposing bacteria after 20 or 45 days.

3 (*b*). *Do.* + *straw.* No cellulose decomposing bacteria after 20 days. After 45 days three tubes inoculated with soil suspension diluted 1:1000 showed two tubes with development of *Spirochaeta cytophaga.*

4 (*a*). *Hoosfield soil, pH* 6·2 + *filter-paper.* After 20 days:

Dilution	Tube no.	Development of micro-organisms
1:10,000	*a* *b*	Paper broken, yellow
1:100,000	*a*	Paper broken, yellow
	b	Sterile
1:1,000,000	*a* *b* *c*	Sterile
Do. After 45 days:		
1:10,000	*a* *b* *c*	*Spirochaeta cytophaga*
1:100,000	*a*	*Spirochaeta cytophaga*
	b	Paper broken, colourless
	c	Sterile

4 (*b*). ***Same soil + straw.*** After 20 days:

Dilution	Tube no.	Development of micro-organisms
1:10,000	*a* *b*	Paper broken, colourless
1:100,000	*a* *b* *c*	No attack

Do. After 45 days:

Dilution	Tube no.	Development of micro-organisms
1:100,000	*a*	*Spirochaeta cytophaga*
	b	Sterile
	c	,,

5 (*a*). ***Garden soil, pH*** 7·1 + ***filter-paper.*** After 20 days:

Dilution	Tube no.	Development of micro-organisms
1:100,000	*a* *b* *c*	Paper broken, colourless
1:1,000,000	*a*	Paper broken, colourless
	b	No attack

5 (*b*). ***Same soil + straw.*** After 20 days:

Dilution	Tube no.	Development of micro-organisms
1:100,000	*a*	Paper broken, colourless
	b	,,
	c	No attack
	d	,,

Do. After 45 days:

Dilution	Tube no.	Development of micro-organisms
1:100,000	*a*	*Spirochaeta cytophaga*
	b	No attack
	c	,,

This experiment thus gave the same general results as the main experiment: at *p*H 5·9 there was only a very slight development of *Spirochaeta cytophaga*, at *p*H 6·2 this organism became more active, and at *p*H 7·1 the vibrios prevailed. At lower *p*H values only the fungi appeared to be active. Plate counts of the fungi showed an abundance of Penicillia, Trichodermae and *Monosporium* in the first three soils, besides *Mucor Ramannianus* in straw treated soils. In the last two soils, especially with addition of straw, the development of the fungi was less abundant, and the most prevalent forms were *Monosporium* sp., *Mycogone nigra*, *Stachybotrys* sp., and an organism resembling *Coccospora agricola* described by Goddard (7).

Second control experiment.

Soil	*p*H	Addition
Clay soil, Cheshire	4·3	1 gm. filter-paper + 21 mg. N as $NaNO_3$ per 100 gm. soil
Sand soil, Woburn	5·7	
Garden soil, Rothamsted	7·1	

Results of the dilution method after 45 days' incubation at 25° C.:

Soil	Dilution	Tube no.	Development of micro-organisms
Cheshire soil	1:100	*a*	Sterile
		b	
		c	
Woburn soil	1:100,000	*a*	*Spirochaeta cytophaga*
		b	
		c	
		d	
Garden soil	1:10,000,000	*a*	Paper broken, colourless
		b	"
		c	"
		d	*Spirochaeta cytophaga*

Here again *Spirochaeta cytophaga* developed in the faintly acid Woburn soil, the reaction of which changed during the experiment to *p*H 6·6, and the vibrios preponderated in the neutral soil. The microscopical appearance of the cultures where the paper was broken at the level of the solution was the same in both the control experiments and in the main experiments: numerous small vibrios, of which one organism, termed *Vibrio* G.St., was isolated from garden soil + straw.

Table I. *Growth of cellulose-decomposing bacteria at varying hydrogen ion concentration.*

	Growth at *p*H*							
Organism	7·6	7·1	6·8	6·4	6·0	5·6	5·2	4·6
Vibrio prima (Kalninš)	+	+	+	+	–	–	–	–
	(2)	(2)	(4)	(6)				
Vibrio napi (Kalninš)	+	+	+	+	–	–	–	–
	(3)	(3)	(4)	(5)				
Vibrio pericoma (Kalninš)	+	+	+	–	–	–	–	–
	(10)	(10)	(20)					
Vibrio bulbosa (Kalninš)	+	+	+	–	–	–	–	–
	(10)	(10)	(20)					
Vibrio agarliquefaciens	+	+	+	+	–	–	–	–
	(4)	(5)	(7)	(12)				
Vibrio B.M.	+	+	+	+	–	–	–	–
	(3)	(3)	(5)	(5)				
Vibrio W. 6	+	+	+	+	–	–	–	–
	(3)	(3)	(3)	(4)				
Vibrio H.M.	+	+	+	+	–	–	–	–
	(4)	(4)	(4)	(5)				
Vibrio G.St.	+	–	+	+	–	–	–	–
	(4)		(8)	(10)				
Vibrio from tap-water	+	+	+	+	+	+	+	–
	(2)	(3)	(3)	(3)	(6)	(20)	(20)	
Spirochaeta cytophaga, impure from Woburn soil	+	+	+	+	+	+	–	–
Spirochaeta cytophaga, pure	+	+	+	+	+	–	–	–

* Figures in brackets indicate number of days which elapsed before paper was broken. Two or three parallel cultures, incubated at 25° C. for 30 days.

A third control experiment was carried out with some soils from abroad: viz. three Gold Coast soils, of *p*H 4·8, 5·2 and 5·9; two Tchernozem soils from Bessarabia, of *p*H 6·6 and 7·4; and an alkali soil from Sudan, of *p*H 9·2. Addition of cellulose and ammonium sulphate gave rise to an abundant development of Penicillia and Trichodermae in the first three soils. In both the Tchernozem soils there was a strong development of *Mycogone nigra*, in that with a reaction of *p*H 7·4 there was also a growth of cellulose-decomposing vibrios, this being the only soil of the series in which cellulose decomposing bacteria appeared active. In the alkali soil there was a fairly strong development of *Stachybotrys* sp.

This close correlation between soil reaction and types of cellulose-decomposing bacteria made it seem desirable to test the ability of the various bacteria to grow at different degrees of acidity. The vibrios mentioned above and a not quite pure culture of *Spirochaete cytophaga* from Woburn soil were grown on filter-paper strips in test-tubes with the mineral nutrient solution mentioned above, p. 82, in which the 0·05 per cent. K_2HPO_4 was replaced by 0·2 per cent. of a mixture of varying proportions of KH_2PO_4 and K_2HPO_4:

Buffer mixture	*p*H after sterilisation
0·2 % KH_2PO_4	4·6
0·008 % K_2HPO_4 + 0·192 % KH_2PO_4	5·2
0·02 % K_2HPO_4 + 0·18 % KH_2PO_4	5·6
0·04 % K_2HPO_4 + 0·16 % KH_2PO_4	6·0
0·1 % K_2HPO_4 + 0·1 % KH_2PO_4	6·4
0·12 % K_2HPO_4 + 0·08 % KH_2PO_4	6·8
0·16 % K_2HPO_4 + 0·04 % KH_2PO_4	7·1
0·2 % K_2HPO_4	7·6

For comparison, the following authentic cultures of cellulose decomposing bacteria were included in the experiment: *Spirochaeta cytophaga*, a pure culture kept for several years in the laboratory. *Vibrio agar-liquefaciens*, freshly isolated from garden soil. *Vibrio prima*, *napi*, *bulbosa* and *pericoma* (13) received from Dr A. Kalninš, University of Riga, Latvia. In addition, a vibrio-like organism, found as accidental infection in tubes with filter-paper and probably coming from tap-water, was included. The results are shown in Table I.

The experiment shows plainly that the vibrios from the soil as well as Kalninš' organisms are very sensitive to acidity, since they fail to develop in the *p*H interval 6·0–6·4. *Spirochaeta cytophaga*, especially the freshly obtained, impure culture, is a little more resistant. These facts agree perfectly with the relative prevalence of these organisms in the soils of different reaction, as shown by the dilution method. While these experiments were in progress there appeared two papers by

Dubos (4, 5), whose results agree with the present ones. When filter-paper or straw was added to soil of different reaction, a multiplication of cellulose-decomposing bacteria took place only at *p*H values not lower than 6·0, and most strongly at *p*H 7·0–8·0. Two cellulose-decomposing vibrios failed in pure culture to develop in the *p*H interval 6·0–6·5; of two other organisms one behaved similarly, and one was still able to grow at *p*H 6·0. *Spirochaeta cytophaga* would, when low concentrations of N were used, grow at *p*H 5·2–5·7. The *Vibrio* from tap-water studied here is considerably more resistant to acidity, but it does not seem active in the soil, since in that case it would probably have been noticed in the dilution experiments, being easily recognised by its formation of a rust-brown pigment in the paper.

II. ACTION OF VARIOUS ORGANISMS ON CELLULOSE IN PURE CULTURE.

The comparative cellulose-decomposing power of the various organisms obtained from the above-mentioned experiments was tested by growing them on pure quartz sand with addition of some cellulosic material and mineral nutrient solution. 150 gm. portions of sand were placed in round 300 c.c. flasks stoppered with tight-fitting perforated rubber stoppers, in which the holes were filled with cotton-wool. At the end of the period of incubation the sand was dried, and cellulose was determined. In the case of pure cellulose this was done by means of the modification of Charpentier's (2) method used by Gray and Chalmers (8): 20 gm. of sand were shaken for 1 hour with 100 c.c. of Schweitzer's solution, the mixture was allowed to settle overnight in a glass cylinder, 50 c.c. were pipetted off, and cellulose was precipitated by addition of an excess of 10 per cent. HCl, filtered off on a dried and weighed filter, washed, dried for 20–24 hours at 35° C., and weighed. For determinations of cellulose in straw, the method of Waksman and Tenney (30) was used: 20 gm. of dried sand were heated in the autoclave for 30 min. with 50 c.c. of a 5 per cent. NaOH solution, the liquid was poured off, extraction repeated, and the residue filtered off and boiled for 30 min. in an approximately 2 per cent. solution of sulphuric acid. After filtering and washing, the sand was dried and extracted with Schweitzer's reagent as before. The figures given in Tables II–V represent the means of duplicate determinations.

First experiment.

Sand + 2 *per cent. ground oat straw* + 1 *per cent.* $CaCO_3$ + 15 *per cent. nutrient solution* ($(NH_4)_2SO_4$ 1 *per cent.*, KH_2PO_4 0·2 *per cent.*, $MgSO_4$ 0·1 *per cent.*, *NaCl* 0·1 *per cent.*).

The following organisms were studied: *Trichoderma* sp. from acid Park plot soil + manure + straw. *Aspergillus fumigatus* from fermenting straw. *Botryosporium* sp. from neutral Park plot soil + manure. *Vibrio* B.M. from the same soil. The first two fungi were also tested in culture without $CaCO_3$. Cultures were incubated for 45 days at 25° C., except the cultures of *Aspergillus fumigatus*, which were incubated at 38° C., the optimal temperature for this organism. The results are shown in Table II.

Table II. *Decomposition of cellulose of oat straw in sand culture.*

Culture	% of straw-cellulose decomposed
Trichoderma sp.	62·1
Do. + 1·0 % $CaCO_3$	10·4
Aspergillus fumigatus	75·9
Do. + 1·0 % $CaCO_3$	79·3
Botryosporium (2) sp. + 1·0 % $CaCO_3$	100·0
Vibrio B.M. + 1·0 % $CaCO_3$	63·8
Control	—
Do. + 1·0 % $CaCO_3$	—

The organisms were all capable of decomposing the cellulose of untreated straw, and the *Vibrio* was as active as *Trichoderma* sp. The activity of the latter organism was strongly reduced by the addition of lime (which agrees with the marked prevalence of this organism during cellulose decomposition in acid soil), whereas *Aspergillus fumigatus* was not affected by the change in reaction. The *Botryosporium* displayed a very strong activity.

Second experiment.

Sand + 1 *per cent. finely cut filter-paper* + 15 *per cent. nutrient solution.* ($NaNO_3$ 0·3 *per cent.*, K_2HPO_4 0·1 *per cent.*, $MgSO_4$ 0·05 *per cent.*, *NaCl* 0·05 *per cent.*).

Pure cultures of the following organisms were tested—all the vibrios isolated in the previous experiment, *Vibrio napi* and *prima* from Dr Kalnins, a pure culture of *Spirochaeta cytophaga*, and the following fungi: *Trichoderma* sp., *Botryosporium* sp., *Monosporium* sp. from Woburn soil, *Penicillium* sp. from Cheshire soil, *Mycogone nigra*, *Coccospora agricola* (?) and *Stachybotrys* sp. from garden soil. Cultures were incubated at 25° C. for 30 days. The results are found in Table III.

Table III. *Decomposition of cellulose as filter-paper in sand + neutral mineral salt solution, by bacteria and fungi.*

Culture	% of added cellulose decomposed	NO$_3$-N mg. per 100 gm. of sand	N resorbed (mg.)	Ratio cellulose decomposed: N resorbed
Vibrio napi (Kalnins)	34·7	0·0	11·4	30:1
Vibrio prima (Kalnins)	40·0	0·0	11·4	33:1
Vibrio B.M.	34·7	0·0	11·4	30:1
Vibrio H.M.	51·6	0·0	11·4	43:1
Vibrio W. 6	35·8	0·0	11·4	30:1
Vibrio G.St.	33·7	0·0	11·4	27:1
Vibrio from water	60·0	0·0	11·4	50:1
Spirochaeta cytophaga	55·8	0·0	11·4	47:1
Trichoderma sp.	0	10·9	(0)	—
Penicillium sp.	(0)	—	—	—
Coccospora sp. (2)	36·8	0·0	11·4	30:1
Monosporium sp.	30·3	0·0	11·4	25:1
Mycogone nigra	64·2	0·0	11·4	54:1
Botryosporium sp. (2)	57·9	0·0	11·4	48:1
Stachybotrys sp.	58·9	0·0	11·4	49:1
Control	—	11·4	—	—

Trichoderma and *Penicillium* were quite inactive here (the reaction was unfavourable, and nitrate is a poor source of N to them), but all the other organisms used up all the available N and decomposed rather unequal amounts of cellulose. Waksman and Heukelekian (9, 28) state that fungi decompose 30–35 units of cellulose for every unit of assimilated N. In the present experiment this was the case only with *Coccospora* sp. and *Monosporium* sp., whereas *Mycogone*, *Botryosporium* and *Stachybotrys* showed a considerably wider ratio, decomposing 48–54 units of cellulose for every unit of N. Waksman (27) mentions in a later communication that *Humicola* sp., an organism described by Traaen (25) and possibly identical with *Mycogone nigra*, shows a similar relationship. The nitrogen requirements of the bacteria were by no means smaller than those of the fungi, as was also suggested by Dubos (4). Indeed, *Vibrio napi*, *prima*, B.M., W.6, and G.St. showed much the same cellulose : nitrogen ratio as found by Waksman and Heukelekian for the fungi, whereas the ratios for *Vibrio* H.M., *Spirochaeta cytophaga*, and the tap-water vibrio were wider, corresponding to *Spirochaeta cytophaga* and *Humicola* sp. according to Waksman (27). It cannot, therefore, be a general rule that the bacteria consume less nitrogen than the fungi, as far as cellulose decomposition is concerned. In the case of *Spirochaeta cytophaga* Hutchinson and Clayton (10) found a cellulose : nitrogen ratio considerably narrower (27–30 : 1) than that observed here. The explanation may be that the amount of N was insufficient in the present experiments, so that some N from dead cells may have been utilised over again.

Also no direct determinations of N were made in Hutchinson and Clayton's experiments.

THIRD EXPERIMENT.

Sand + 1 per cent. finely cut filter-paper + 15 per cent. mineral salt solution (($(NH_4)_2SO_4$ 1 per cent., KH_2PO_4 0·2 per cent., $MgSO_4$ 0·05 per cent., NaCl 0·05 per cent.).

Only the fungi were included in this experiment. Cultures were incubated for 30 days at 25° C. The results are shown in Table IV.

Table IV. *Decomposition of cellulose as filter-paper by fungi in sand with acid mineral salt solution.*

Culture	% of added cellulose decomposed	NH_4-N mg. per 100 gm. of sand	N resorbed (mg.)	Ratio cellulose decomposed: N resorbed
Trichoderma sp.	27·4	21·1	6·9	37:1
Penicillium sp.	20·0	23·6	4·4	44:1
Coccospora sp.	14·6	23·4	4·6	30:1
Monosporium sp.	26·5	22·1	5·9	44:1
Mycogone nigra	13·7	25·1	2·9	45:1
Botryosporium sp.	(0)	27·8	(0)	—
Control	—	28·0	—	—

There was less cellulose decomposition than in the previous experiment. *Trichoderma* and *Monosporium* were the most active organisms here. The activity of *Mycogone* and *Coccospora* was greatly diminished owing to the acidity reaction, and *Botryosporium* was rendered quite inactive. It is noteworthy that only *Trichoderma* and *Coccospora* showed approximately the cellulose:nitrogen ratio found by Waksman and Heukelekian for the fungi, whereas the three others showed a wider ratio, although there was here a considerable excess of NH_4-N and consequently no reason to suspect a repeated utilisation of N which was assimilated and again released, such as might have occurred in the previous experiment, where the whole supply of N was used up.

FOURTH EXPERIMENT.

Sand + 1 per cent. crude[1] *lignocellulose + 1 per cent. $CaCO_3$ + 15 per cent. of the same solution as in the second experiment.*

Cultures were incubated for 30 days at 25° C. Results are found in Table V.

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[1] Prepared from ground oat straw by boiling for 1 hour with 2 per cent. H_2SO_4, filtering, washing till free from acid, and drying.

Table V. *Decomposition of cellulose of acid-extracted oat straw in sand with neutral mineral salt solution, by bacteria and fungi.*

Culture	% of added cellulose decomposed	NO_3-N mg. per 100 gm. of sand	NO_3-N resorbed (mg.)
Vibrio B.M.	57·9	0·0	11·4
Vibrio H.M.	50·0	0·0	11·4
Vibrio W. 6	47·4	0·0	11·4
Vibrio G.St.	57·9	0·0	11·4
Vibrio from water	68·4	0·0	11·4
Coccospora sp.	63·2	0·0	11·4
Mycogone nigra	63·2	0·0	11·4
Botryosporium sp.	86·8	0·0	11·4
Stachybotrys sp.	81·8	0·0	11·4
Control	—	11·4	—

All the cellulose-decomposing vibrios as well as the fungi were capable of decomposing the lignified cellulose. *Stachybotrys* and *Botryosporium* were most active. The cellulose:nitrogen ratio appeared wider here than in the case of pure cellulose probably because the acid-treated straw still contained some proteid material, which may have served as nitrogenous food.

In some of the sand cultures of fungi on pure cellulose the medium became dark coloured at the end of the experiment. This suggested the formation of humic substances from the cellulose, a much discussed question which cannot be reviewed in detail here, but which may have a bearing on the formation of resistant nitrogenous humus during the decomposition of manure on the soil, as shown in a previous paper (12). A qualitative test for *a*-humus was made by extracting 20 gm. of dry sand (from experiments 2 and 3) with 20 c.c. 2·5 per cent. NaOH, in the autoclave, filtering, and acidifying the extract with HCl.

Table VI. *Production of humus-like substances by cellulose-decomposing organisms in sand culture.*

Organism	Colour of NaOH-extract	Precipitate with HCl
Vibrio prima	Pale yellow	None
Vibrio napi	”	”
Vibrio B.M.	”	”
Vibrio W. 6	”	”
Vibrio H.M.	”	”
Spirochaeta cytophaga	Golden	”
Vibrio from water	Light yellowish brown	”
Vibrio G.St.	Pale yellow	”
Coccospora sp.	Yellowish brown	”
Monosporium sp.	”	”
Trichoderma sp.	Light brown	Trace, brownish
Botryosporium sp.	Yellowish brown	Trace, yellowish
Mycogone nigra	Coffee brown	Brown, flocculent
Stachybotrys sp.	Purplish black	Black, flocculent

The data in Table VI show that the cellulose-decomposing bacteria did not form any such substances (as was also found by du Toit (6)), and the same is the case with some of the fungi, but two of these, *Mycogone* and *Stachybotrys*, formed a detectable amount of matter possessing the external characters of α-humus, or humic acid: an amorphous, dark brown to black material, soluble in alkalies and precipitated by acids. Enough sand-cellulose mixture was available to make a quantitative analysis. This gave the results shown in Table VII.

Table VII. *Formation of humus-like substances by fungi in sand culture.*

Organism	Sand extracted (gm.)	Humus obtained (gm.)	% of N in humus
Mycogone nigra	70	0·016	4·0
Stachybotrys sp.	90	0·036	5·9

The percentage of N in the humus-like material was thus of the same order as in soil humic acid. The fungi also form humus when growing in sterilised soil, as shown by another experiment. A light sand soil from Tunstall, of *p*H 4·9, received additions of 2 per cent. filter-paper, a source of N, and water to bring the soil up to 75 per cent. of its water-holding capacity. Portions of 150 gm. were placed in round 300 c.c. flasks, sterilised, and inoculated with four cellulose-decomposing fungi. The following series was run:

Addition besides filter-paper	Inoculation
Nothing	Sterile (control)
0·25 % $(NH_4)_2SO_4$	*Trichoderma* sp.
	Mycogone nigra
0·25 % $NaNO_3$ + 1·0 % $CaCO_3$	*Stachybotrys* sp.
	Botryosporium sp.

After 50 days' incubation at 25° C. the results shown in Table VIII were obtained.

It is seen that *Trichoderma* and *Botryosporium*, which did not produce significant quantities of humus in sand, gave only a very slight increase in the amount of α-humus, but a considerable increase in its N percentage. This is probably due to the extraction of some protein from the mycelium. *Mycogone nigra* gave a distinct increase in the amount of α-humus, and its N percentage shows less increase (the increase corresponds to a content of 5 per cent. N in the extra humus which the fungus has formed). Finally, *Stachybotrys* gave a very notable increase in the amount of α-humus and a corresponding increase in its N percentage. This experiment with pure cultures confirms the statement of Waksman (27) that a nitrogenous α-humus can be formed from pure cellulose decomposing in sand, and offers us an explanation of the extra formation of α-humus during manure decomposition in soil, since the two fungi studied here are active in the decomposition of straw in neutral and alkaline soils. The humus formation is not, however, necessarily dependent on the presence of cellulose, since the humus-like material is a constituent of the protoplasm of these and other micro-organisms and can be formed from other carbonaceous food. This leads us to the problem of humus formation through the decomposition of microbial protoplasm, which will be dealt with in another contribution.

Table VIII. *Production of α-humus by pure cultures of cellulose-decomposing fungi in sterile soil.*

Organisms	α-humus (%)	N in α-humus (%)*	Appearance of cultures
Control, sterile	0·21	3·39	—
Trichoderma sp.	0·25	4·47	After 1–3 weeks' abundant mycelial development and sporulation, after 4–6 weeks' growth hardly visible
Mycogone nigra	0·28	3·84	After 1–3 weeks' strong mycelial development, which later disappears
Stachybotrys sp.	0·40	4·20	Very little mycelial development, but soil coloured dark by spores
Botryosporium sp.	0·23	4·41	Visible mycelial growth, but less strong than that of *Trichoderma* and *Mycogone*

* Average of two parallel determinations. Method of humus determination described in the previous paper.

SUMMARY.

1. Addition of farmyard manure to soil gives rise, in laboratory experiments, to an abundant development of cellulose-decomposing bacteria of the genus *Vibrio* in approximately neutral soils (pH 6·5–7·0). In faintly acid soils (pH 5·7–6·2) these organisms develop less abundantly, and are partly replaced by *Spirochaeta cytophaga*. At lower pH values only the fungi are active in the decomposition of cellulose. Similar results were obtained by adding filter-paper or straw to soils of different reactions. Of the fungi, *Trichoderma* and *Penicillium* appear more active in acid soil, whereas other forms, among others *Mycogone nigra*, *Stachybotrys* sp., *Coccospora agricola* (?), and *Botryosporium* sp. seemed prominent in neutral soil.

2. The vibrios, of which four strains were studied in pure culture, are very sensitive to acidity. They fail to develop in the pH interval

6·0–6·4, and have an optimum at *p*H 7·1–7·6. *Spirochaeta cytophaga* appears to be slightly more resistant to acidity, being able to grow at *p*H 5·6–6·0.

3. The bacteria as well as the fungi are capable of decomposing the lignified cellulose of straw.

4. The nitrogen requirements of the cellulose-decomposing bacteria are not smaller than those of the fungi. The ratio of decomposed cellulose to assimilated nitrogen in pure cultures ranges between 25:1 and 54:1 without any clear difference between the two groups of organisms.

5. Cellulose-decomposing bacteria do not form humus-like compounds when growing on filter-paper in sand culture, but at least two of the fungi, *Mycogone nigra* and *Stachybotrys* sp., form such compounds in sand as well as in sterile soil.

The writer wishes to express his most sincere thanks to Sir John Russell, D.Sc., F.R.S., Director of the Rothamsted Experimental Station, for placing the facilities of the laboratories at the writer's disposal, to Dr H. G. Thornton, under whose guidance the work was carried out, and to Mr P. H. H. Gray, M.A., for much kind assistance during the course of the work.

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THE MICROBIOLOGY OF FARMYARD MANURE DECOMPOSITION IN SOIL.

III. DECOMPOSITION OF THE CELLS OF MICRO-ORGANISMS.

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(With Seven Text-figures.)

Introduction.

In the two previous papers of this series (22, 23) it was shown that addition of manure to soil gives rise to a rapid multiplication of bacteria, of actinomycetes and sometimes of filamentous fungi, which last develop to an enormous extent in acid soils, if the manure contains fresh straw. There is also an increase in cellulose-decomposing organisms. Until this growth rate diminishes, no considerable accumulation of ammonia or nitrate occurs, probably because a large proportion of the available N of the manure is assimilated by the micro-organisms and is not nitrified until after the death and subsequent decomposition of their cells. This renders it a matter of interest to study the decomposition of dead microbial protoplasm in the soil—a matter about which we know very little.

It has been repeatedly suggested that the resistance to decomposition of bacterial substance may be responsible for the difficulty with which the organic N of farmyard manure is utilised by plants, it being claimed that bacterial and fungal cells account for a very considerable proportion thereof. Ehrenberg (12) assumes that the N assimilated in fungal protoplasm, especially spores, is in the most resistant state that can be imagined. Löhnis (28) compares the fertilising value of the microbial matter with that of leather meal, a view which is shared by Ruschmann (33), who even speaks of "der notorischen Unzersetzlichkeit der Bakteriensubstanz." Also Barthel and Bengtsson (1) seek to explain the absence of nitrification of the organic N of manure in laboratory experiments through the resistance of the N-compounds of bacterial cells, especially nuclein compounds which are also present in epithelial cells from the digestive tract of animals.

The presumption that nucleic compounds are resistant is probably based upon the fact that Stutzer (37) found about 40 per cent. of the N of fungal protoplasm represented by nucleic compounds, and that Stutzer and Klingenberg (38) found inferior nitrogenous fertilisers, such as leather meal, horn meal, and wool dust, very rich in pepsin-indigestible N compounds, probably nucleins. It must be admitted that our knowledge of the decomposition of nucleic compounds is incomplete, but the scanty evidence at hand does not seem to indicate a great resistance to decomposition. Fungi (Iwanoff (20)) as well as a number of bacteria are capable of decomposing nucleic acids (Schittenhelm and Schröter (34), Plenge (31)), as well as nucleoproteids (Koch and Oelsner (24)). Both nucleic acids and purin derivates undergo nitrification when added to the soil (Funchess (15), Batham (3)). The N in nucleins is present in the form of purin derivatives.

As to the decomposition of the bacterial substance as a whole, it has been known for a very long time that bacteria can be completely dissolved by substances produced by themselves (autolysis) or by other bacteria. The classical example of this is the so-called pyocyanase (Emmerich and Löw (13)), which is present in old broth cultures of *Pseudomonas pyocyanea* and is capable of bacteriolysing a number of other bacteria. Ruschmann (33) observed a similar cytoclastic activity of *Bac. vulgatus* and *mycoides* towards *Micrococcus candicans* and *pyogenes*. He ascribed the value of the "Edelmist"-process to the high-temperature fermentation killing the vegetative cells of the bacilli, while allowing their enzymes to act upon the other dead bacterial cells, whose N-compounds are thus rendered soluble. Lieske (26) states that several actinomycetes are capable of exerting a strong cytoclastic activity towards many species of bacteria. Digestive enzymes are also to some extent capable of dissolving bacterial proteins; Kruse (25) states that living cells seem quite resistant, whereas heating or treatment with antiseptics render the Gram-negative bacteria digestible, but the Gram-positive ones are less easily made digestible by those treatments. Treatment with fat solvents has a similar effect (Dukes (11)). Beijerinck and van Delden (4) found a considerable nitrate formation in old crude solution cultures of *Azotobacter chroococcum*. Bonazzi (8), on the other hand, found the proteins of this organism very little digestible. An excellent contribution explaining the controversy has been furnished by Molér (29) who found that *Azotobacter chroococcum* does not form any soluble N compounds in pure cultures, even very old ones, and cannot be attacked either by pyocyanase or by *Ps. fluorescens*. In crude cultures, however, soluble N-compounds are formed, probably through the activity of amoebae, which regularly accompany *Azotobacter* in crude cultures and are capable of digesting its cells. Apart from some observations on autolysis in pure cultures by Dox and Maynard (10), Brenner (9), and Waksman (41), as to the digestion of fungus protoplasm, we have but little information, and none at all concerning the third large group of soil micro-organisms, viz. the actinomycetes.

The decomposition of microbial matter *in soil* has been, as mentioned above, very little studied, and it appears doubtful whether the results of the digestion experiments, carried out at rather high temperatures and for a few days at the most, really apply to the conditions in the soil where the material is exposed to the attack of many kinds of micro-organisms. Müller (30) noticed an abundance of dark, coarse fungus hyphae, apparently extremely resistant to decomposition, in forest soils of peat type, whereas hyaline, easily destructible hyphae prevailed in soils of "mould" type. The first chemical experiment in this direction was carried out by Bierema (7), who found that 0–28 per cent. of the N of various bacteria was nitrified in soil after 2 months; 42 per cent. of the N of *Mucor racemosus* was nitrified in the same time, whereas N of *Penicillium glaucum* was nitrified only slightly or not at all. Unfortunately, the composition of these various kinds of substance was not stated, apart from the total amounts and percentages of N. Honcamp (19) found 72 per cent. of the N of dry yeast transformed into ammonia during 8–9 weeks in sand soil; Starkey (35) found that the decomposition of mixed fungus material in soil, measured by the CO_2 evolution, was as rapid as that of lucerne meal. Waksman (42) found a fairly rapid production of ammonia and nitrate from mycelium of *Aspergillus niger* and *Trichoderma* sp. in sand and soil in 45 days, and the author (21) observed the same of *Polyporus* sp. in garden soil, whereas Rege (32) found the N in mycelium of *Coprinus fimetarius* very little available to mustard plants in pot experiments.

While the experiments described in the present contribution were in progress, there appeared two more interesting papers bearing upon this subject. Barthel and Bengtsson (2) found that the mycelium of *Aspergillus niger* underwent quite rapid nitrification in soil, about 41 per cent. of its N being transformed into nitrate in 2 months. Sterilisation of the mycelium seemed to retard the rate of nitrification. Similar experiments were carried out with dried cell material of *Urobac. Pasteurii*, *Bac.*

radicicola, and *Azotobacter chroococcum*. The following results were found:

Organism	% N in dry substance	% N nitrified after 2 months	4 months	(C : N ratio*)
Urobac. Pasteurii	11·41	60·7	64·9	4·0 : 1
Bac. radicicola	3·90	34·1	33·8	11·5 : 1
Az. chroococcum	1·63	None	None	27·6 : 1

* Calculated by the author, assuming 45 per cent. C in the dry substance.

Barthel and Bengtsson thought that the absence of nitrification of *Azotobacter* substance denoted a particular resistance of this material; but when the approximate carbon: nitrogen ratios are calculated as shown above, the results are seen to agree perfectly with those obtained by the author in another paper (21), and the difference in the degree of nitrifiability seems to be accounted for by the difference in carbon: nitrogen ratio. This important factor has been taken into consideration in the other paper by Heck (16), whose very complete investigation shows that fungus material (fruit bodies of various higher fungi, and mycelium of *Aspergillus*, *Trichoderma* and *Coprinus*) is on the whole as rapidly decomposed in soil as is other organic material (blood meal, cotton-seed meal, lucerne-seed meal, water-insoluble fractions of the same materials, timothy hay, and straw) of similar C:N ratio. A nitrification test for 80 days in soil showed a good correlation between C:N ratio (ranging from 3·2:1 in blood meal to 45·7:1 in straw) and degree of nitrification[1]. Materials with C:N ratio 20:1 or more produced very little or no nitrate in this time. Also the quality of the energy material was of importance; when much cellulosic material was present the decomposition seemed to be carried out largely by fungi, and a smaller accumulation of nitrate resulted.

Another point of interest in the decomposition of microbial substance is the formation of resistant humus-like nitrogenous compounds. Müller (30) suspected the fungi of peat soils (*e.g. Cladosporium humifaciens*) to be the agents of humus formation, and the opinion that the resistant N-compounds of the humus are the remains of fungus mycelium has also been expressed by Beijerinck (5) and more recently by Süchting (39). That microbial protoplasm can serve as a source of soil humus was demonstrated by Trussov (40), and the question was later studied by

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[1] A calculation of the correlation coefficient (r) between C : N ratios and percentage of nitrified N from Heck's data (eighteen kinds of fungus material, seven kinds of other plant material) shows $r = -0{\cdot}715$, a value of very high significance. Only one sort of material—mycelium of *Pholiota* sp.—departed from the normal, showing no nitrification after 80 days in spite of a C : N ratio of 10·9 : 1.

Waksman (42) who demonstrated the presence of a nitrogenous material, apparently possessing the properties of "α-humus" or "humic acid" in mycelium of *Asp. niger* and *Trichoderma* sp. The author (21) found a similar substance in the mycelium of *Polyporus* sp., and in another paper (23) he showed that similar materials were produced by two cellulose-decomposing fungi, *Mycogone nigra* and *Stachybotrys* sp., active in straw decomposition in neutral and alkaline soils.

In the present contribution a series of decomposition experiments has been carried out with cell material of various fungi, actinomycetes and bacteria, partly in order to obtain information about the rapidity with which their N is again mineralised, partly in order to ascertain whether a resistant nitrogenous "α-humus"-like material is generally left behind, as supposed by Waksman (42).

EXPERIMENTAL.

(1) *Materials used for the decomposition experiments.*

Cell material of the following organisms was used:

A. Soil fungi: *Zygorhynchus* sp. (*Vuilleminii?*); *Trichoderma* sp. (*Koningi?*); *Aspergillus carbonarius*; *Mycogone nigra*; *Stachybotrys* sp.

B. Manure fungi: *Aspergillus fumigatus*; *Coprinus* sp.

C. Wood-destroying fungus: *Polyporus* sp.

D. Actinomycetes: *Actinomyces griseus.*

E. Soil bacteria: Bacterium sp., from acid Park plot soil + manure; *Bac. megatherium,* from garden soil.

The fungi and actinomycetes were grown in suitable nutrient solutions (Czapek's solution for the aspergilli, *Mycogone,* and *Stachybotrys*; dextrose-peptone solution for *Trichoderma, Zygorhynchus, Coprinus,* and *Actinomycetes*) at approximately optimal temperatures and reaction. When a good growth had developed, it was filtered off, dried and ground. The *Polyporus* material was the same as that used in another experiment (21). The bacteria were grown on dextrose-peptone agar in large tubes, from which the mass of growth was scraped off, dried on the water bath and ground. The composition of the various cell materials as well as of a few others, with which they were compared, is presented in Table I.

(2) *Decomposition experiments in soil.*

The soil used for these experiments was a heavy loam, fairly rich in organic matter, from a plot of garden soil at Rothamsted Experimental Station. It was of neutral reaction (*p*H 7·1–7·2). Portions of 400 to

Table I. *Composition of microbial substances and other plant materials.*

Substance	% of air-dry material				C : N ratio
	Total N	Total C	Ash	Water	
Trichoderma sp.	4·91	43·9	4·8	7·4	8·9 : 1
Zygorhynchus sp.	5·01	46·8	12·1	6·5	9·3 : 1
Aspergillus carbonarius	3·75	45·7	1·6	—	12·2 : 1
Aspergillus fumigatus	3·54	41·8	3·7	8·6	11·8 : 1
Extraction residue of *Aspergillus fumigatus*[*]	3·53	44·7	0·4	—	12·7 : 1
Mycogone nigra I	4·94	43·5	6·1	—	8·8 : 1
„ II	5·15	—	—	—	—
Stachybotrys sp. I	4·84	44·8	6·9	—	9·3 : 1
„ II	3·78	—	—	—	—
Coprinus sp.	4·68	39·4	0·0	7·7	8·4 : 1
Polyporus sp.	4·45	45·5	—	—	10·2 : 1
Actinomyces griseus	8·92	42·4	11·6	8·3	4·8 : 1
Bacterium sp.[†]	5·51	—	—	—	—
Bac. megatherium[†]	6·69	—	—	—	—
Lucerne meal	3·46	44·6	—	—	12·9 : 1
Lupin meal	2·26	45·2	—	—	20·0 : 1
Wheat straw	0·33	40·0	—	—	124 : 1

[*] Prepared from the mycelium by thrice-repeated boiling with 2·5 per cent. NaOH solution, filtering, boiling with 2·5 per cent. sulphuric acid, filtering, and washing till free from acid.

[†] Too little material available for carbon determination.

500 gm. of air-dry soil received additions of 1·0 per cent. air-dry microbial substance + 27 per cent. water, and were kept moist at 25° C. for 120 days, during which periods the numbers of bacteria and actinomycetes, and amounts of ammonia and nitrate, were determined at intervals. At the end of the experiment α-humus and N therein were also determined. The methods were the same as described in the first paper of this series (22). Since it was impossible to carry out all the experiments simultaneously, four different samples of soil had to be used, and the experiment was subdivided as follows.

A. Control soil I: soil + *Aspergillus fumigatus*; soil + *Polyporus* sp.[1]

B. Control soil II: soil + *Trichoderma* sp.; soil + *Zygorhynchus* sp.; soil + *Actinomyces griseus*[1]; soil + Bacterium sp.; soil + *Bac. megatherium*.

C. Control soil III: soil + *Coprinus* sp.

D. Control soil IV: soil + *Mycogone nigra* (Preparation II, see Table I); soil + *Stachybotrys* sp. (Preparation II, see Table I).

The plate counts of bacteria and actinomycetes in the four control soils are shown in Figs. 1–3. The numbers were of the same order of magnitude in the three first soils and never exceeded 50 millions of bacteria per gm. of soil. In the fourth soil the numbers were determined only at the start and at the end of the experiment, but there is no reason to suppose that the numbers in this soil differed widely from the three previous ones.

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[1] These two materials were sterilised before use by heating the dry material in the autoclave, in order to make the plate counts possible, since both materials were very rich in spores.

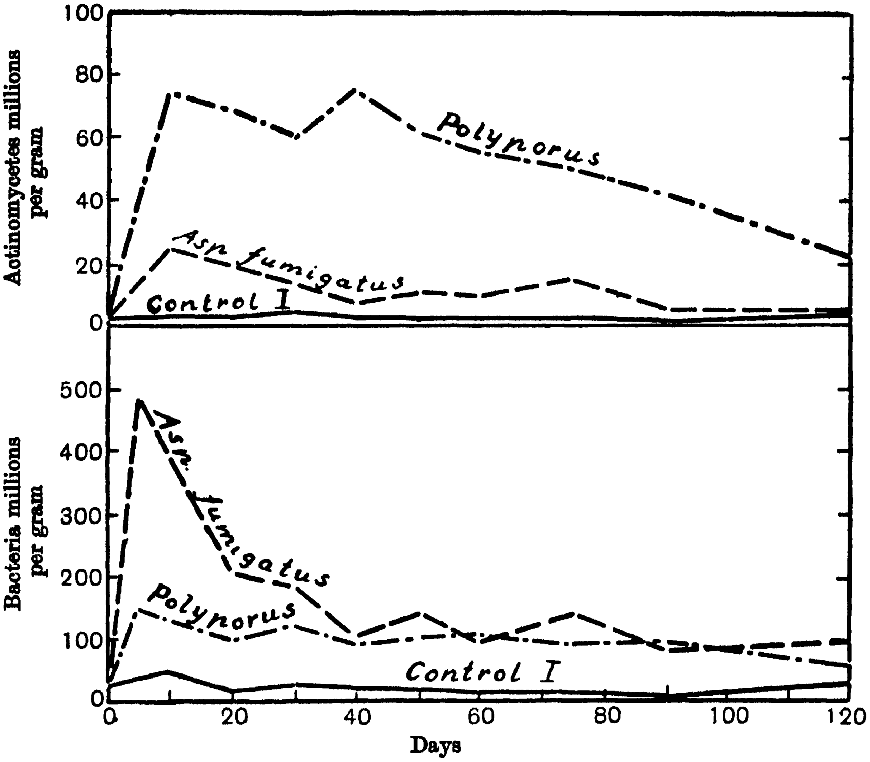

Fig. 1. Multiplication of bacteria and actinomycetes in garden soil with addition of microbial substance.

The numbers of micro-organisms in soils with additions are seen in the same figures. Immediately after the start of the experiment there was in all cases an immense multiplication of the bacteria, which reached their maximal numbers after 5, or occasionally, 10 days. The numbers then fell rather suddenly and gradually became steady, but remained in all cases higher than in the control soils. The highest numbers occurred in soil + *Trichoderma*, the lowest in soil + *Polyporus* and *Stachybotrys*. Only in soil + *Bac. megatherium* were there two maxima, this being due to the fact that in this case a large number of colonies originated from still living spores of the bacillus. The actinomycetes, like the bacteria,

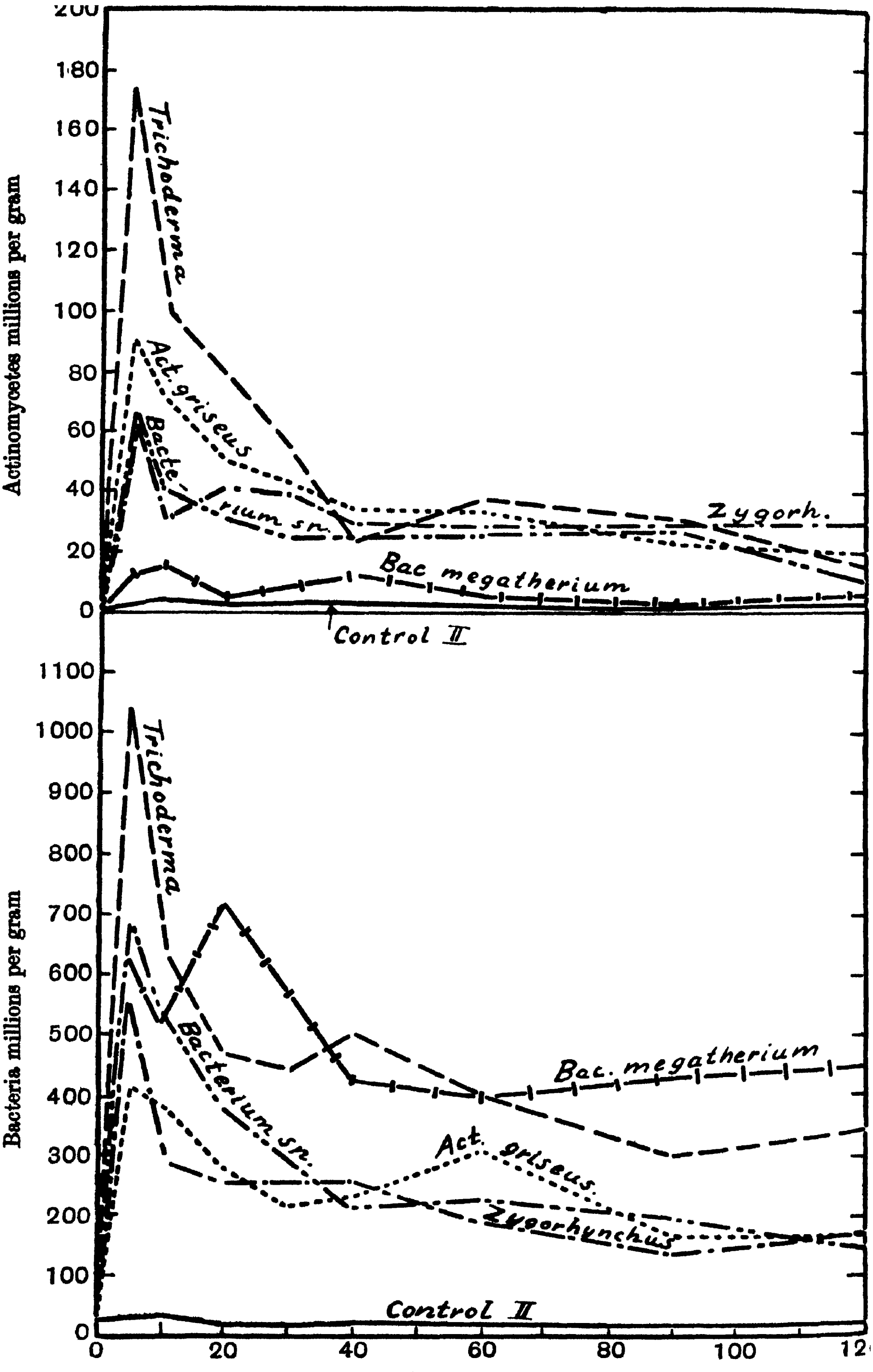

Fig. 2. Multiplication of bacteria and actinomycetes in garden soil with addition of microbial substance.

were stimulated in all cases and reached their maximal numbers in most cases after 5–10 days, after which time their numbers fell again, though not so suddenly as those of the bacteria. The highest actual numbers

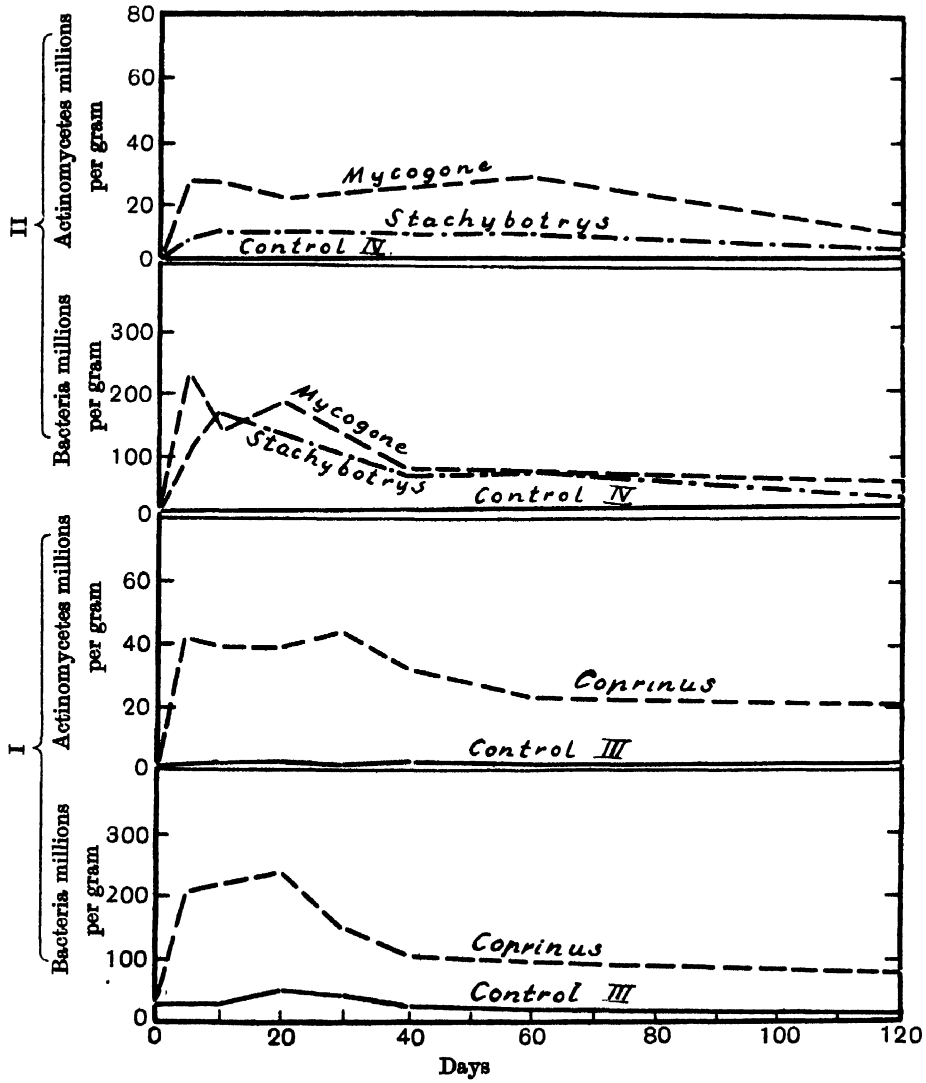

Fig. 3. Multiplication of bacteria and actinomycetes in garden soil with addition of microbial substances.

occurred in soil + *Trichoderma*, but the count was comparatively little influenced by *Stachybotrys* and *Bac. megatherium.* The *relatively* strongest development of actinomycetes took place in soil + *Polyporus*, where the colonies of actinomycetes were more than half as numerous as those of the bacteria. These results upon the whole support the opinion expressed by Waksman and Skinner (43), that one of the chief functions of the actinomycetes in the soil is the decomposition of dead microbial, particularly fungal, substance. This agrees well with the fact that actinomycetes flourish abundantly after partial sterilisation of soil (Hiltner and Störmer (17), Waksman and Starkey (44)).

Besides these determinations, counts of fungi were carried out in the soils of Section A. Only a very slight influence of the fungus material on the abundance of fungi was noticeable.

Table II. *Decomposition of microbial substance in garden soil.*

Soil and addition of microbial substance	% of added N nitrified after		α-humus (%)	N in α-humus (%)
	60 days	120 days		
Control I	—	—	1·36	3·86
„ + *Asp. fumigatus*	29·9	44·3	1·46	3·88
„ + *Polyporus* sp.	23·6	19·3	1·53	4·11
Control II	—	—	1·49	3·76
„ + *Trichoderma* sp.	50·7	49·5	1·46	3·90
„ + *Zygorhynchus* sp.	28·3	27·3	1·50	4·09
„ + *Act. griseus*	60·5	69·3	1·45	4·11
„ + Bacterium sp.	53·8	—	1·49	4·23
„ + *Bac. megatherium*	47·3	53·1	1·39	4·11
Control III	—	—	1·43	4·04
„ + *Coprinus* sp.	36·1	40·8	1·45	4·19
Control IV	—	—	1·44	3·70
„ + *Mycogone nigra*	—	39·7	1·45	3·78
„ + *Stachybotrys* sp.	—	25·9	1·44	4·21

The chemical changes are represented in Figs. 5–8 and Table II. There was in most cases an abundant nitrate production by the 20th day, but no accumulation of ammonia (no reaction with Nessler's solution in a KCl-solution extract). In the next 20–40 days the amounts of nitrate increased somewhat, but not very markedly, while after 60 and after 120 days there was in most cases little further increase, a result which showed that a part of the N of microbial substance is very readily transformed into nitrate, whereas the rest persists in the soil as a very resistant residue. This fact can also be seen from the data of Barthel and Bengtsson (2) cited above, and it holds also for farmyard manure and other organic materials, as previously pointed out by the author (21). The fraction nitrified is rather variable, from 19 to 23 per cent. in *Polyporus* to 61–69 per cent. in *Actinomyces*. The C:N ratio had little influence here; *Act. griseus* with the narrowest C:N ratio showed indeed the strongest nitrification, but *Zygorhynchus* with C:N = 3:1 showed much less nitrification than *Asp. fumigatus* with C:N = 11·8:1. Apparently the unnitrified N was not merely that portion which was assimilated by the agents of decomposition. It seems rather that microbial substances contain unlike quantities of some material which is itself resistant to decomposition. By a previous experiment, this was shown to be the case with *Polyporus* material, which contains a nitrogenous fraction of "humic acid" character. The α-humus determinations in Table II show, that in these experiments also, *Polyporus* gave a significant increase in both the amount and N percentage of soil α-humus. *Aspergillus* also gave a small increase, but the addition of the other kinds of microbial substance did not result in any appreciable formation of α-humus. The N percentage of the humus was a little increased in all

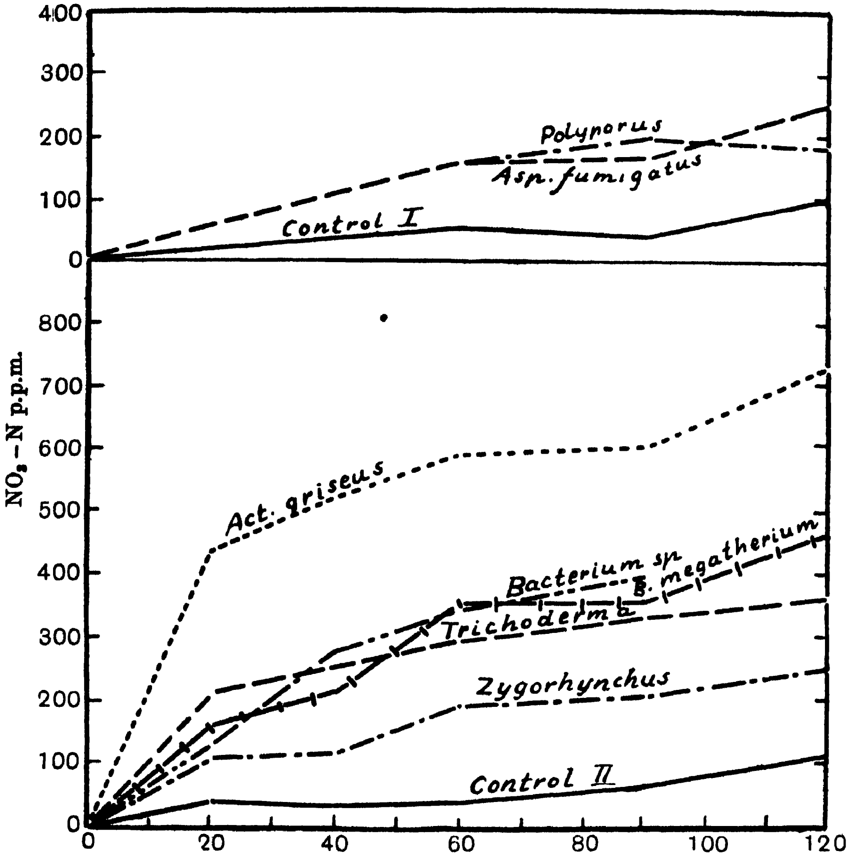

Fig. 4. Production of nitrate from microbial substance (*Aspergillus fumigatus, Polyporus* sp., *Trichoderma* sp., *Zygorhynchus* sp., *Actinomyces griseus*, Bacterium sp., *Bacillus megatherium*) in garden soil.

cases, probably because all the soils with additions were much richer in bacteria at the end of the experiment, and in the α-humus determinations some protein may have been extracted from the bacterial cells. It is at first sight rather surprising, in view of results previously obtained (23), that *Mycogone* and *Stachybotrys* did not give any increase in α-humus. The materials used here were, however, somewhat abnormal partly autolysed mycelia from very old cultures, in which the nutrient solution was coloured deep brown from soluble substances, which could be precipitated by acidification and probably represented the humus-like fraction which had diffused out from the cells. Moreover, the control experiments in sand with a more normal material gave a different result, as shown below.

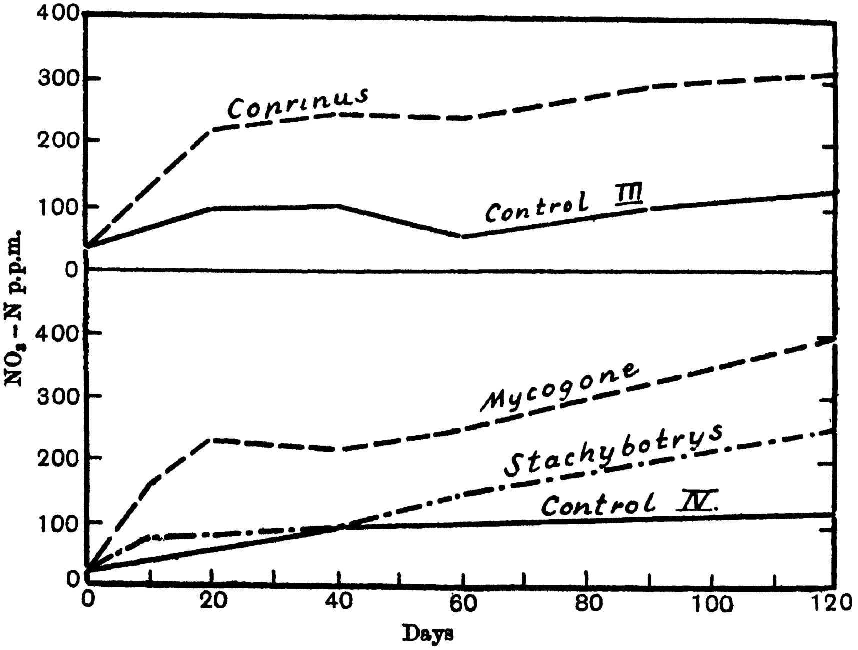

Fig. 5. Production of nitrate from microbial substance (*Coprinus* sp., *Mycogone nigra*, *Stachybotrys* sp.) in garden soil.

(3) *Decomposition experiments in sand.*

The experiments just described are open to the criticism that the soil itself is rather rich in α-humus, so that the formation of a small amount of humus from the added material would be difficult to detect and might easily have escaped determination. To obtain a control upon this and also to determine how far the excess of nitrate over controls represented a true formation of nitrate from the added materials, a series of control experiments was carried out in sand medium, wherein all α-humus and all mineral N must originate from decomposed material and not from the substratum.

250–300 gm. portions of sand received additions of 2·0 per cent. of air-dry microbial substance or of higher plant material (only 1·0 per cent. in the case of *Actinomyces griseus*), 15 per cent. water, and 1 c.c. of a 1:10 suspension of garden soil as an inoculum. All the materials mentioned in Table I were used except the preparations *Mycogone nigra* II and *Stachybotrys* II, and the two bacteria, of which no more material was available. The moist sand mixtures were kept at 25° C. for 90 days, and amounts of ammonia determined at intervals by extracting 10 gm. of the moist sand with 0·5 *N* KCl and distilling the extract with MgO. No nitrate or nitrite could be detected by testing the extract with diphenylamine-sulphuric acid. At the end of the experiment total N, total C, α-humus and N were determined. The results are shown in Table III.

In nearly all cases (except lupin meal, straw and extraction residue of *Aspergillus*) an abundant production of ammonia took place by the tenth day, but later considerable quantities of ammonia were lost through evaporation, so that the ammonia determinations did not here give a reliable measure of the decomposition. A better index of this was given by the determinations of the residual amounts of organic N, which show that most of the fungus materials left a quantity similar to or slightly higher than that of lucerne and lupin meal, but a few materials—*Asp. carbonarius*, *Stachybotrys*, *Polyporus*, and *Act. griseus*—appeared more resistant. The carbon determinations showed that the C:N ratio of the residues of most microbial materials were significantly lower (4·3–8·5:1) than that usually found in the soil (10–12:1). This can be explained by the fact that the soil humus consists partly of matter derived from N-free lignin and partly of nitrogenous matter possibly of microbial origin, whereas this experiment dealt only with the latter. The lucerne, the lupin and the extraction residues of *Aspergillus* mycelium, left residues of C:N ratio similar to that of the soil, and the same was the case with the mixture of straw and *Zygorhynchus* mycelium; it is noteworthy that all these materials, except the extraction residue, contained lignin.

With most of the added materials, the degree of decomposition, expressed as the percentage of mineralised N (*i.e.* total addition of N minus

Table III. *Decomposition of microbial substance in sand medium.*

NH_4-N, mgm. per 100 gm. of dry sand after	*Trichoderma* sp.	*Zygorhynchus* sp.	*Asp. fumigatus*	Extraction residue of *Asp. fumigatus*	*Asp. carbonarius*	*Mycogone nigra*	*Stachybotrys* sp.
10 days	39·5	43·3	11·1	0·0	13·5	31·5	20·2
20 ,,	52·4	60·5	27·4	2·4	17·1	39·5	19·2
40 ,,	59·2	51·6	29·0	7·3	28·8	34·0	15·3
60 ,,	59·2	64·5	28·2	11·5	28·0	28·0	12·2
90 ,,	58·3	59·0	22·3	14·0	22·1	13·4	11·2
Total N after 90 days, mgm. per 100 gm. of sand*	83·0	89·0	57·1	47·3	67·9	48·2	63·6
Organic N after 90 days (Total N — NH_4-N), mgm. per 100 gm. of sand	24·7	30·0	34·8	33·3	45·8	34·8	53·4
Organic C after 90 days (%)*	0·152	0·154	0·256	0·365	0·324	0·286	0·441
C : N ratio (organic) after 90 days	6·2 : 1	5·1 : 1	7·4 : 1	10·9 : 1	7·0 : 1	8·2 : 1	8·4 : 1
Total N added, mgm. per 100 gm. of sand	98·2	100·2	70·8	70·6	75·0	98·8	96·8
% of added N mineralised	74·8	70·0	50·9	52·8	38·9	64·8	45·9
α-humus (%)	None	Trace	0·09	0·06	0·14	0·18	0·31
% N in α-humus	—	—	6·1	7·5	3·6	4·0	4·0

NH_4-N, mgm. per 100 gm. of dry sand after	*Coprinus* sp.	*Polyporus* sp.	*Actinomyces griseus*	Lucerne meal	Lupin meal	Wheat straw	Straw + *Zygorhynchus* sp.†
10 days	40·6	15·5	29·4	33·1	0·8	0·0	33·4
20 ,,	39·5	29·0	33·9	29·0	0·0	0·0	38·4
40 ,,	34·3	32·3	36·3	19·3	3·2	0·0	40·2
60 ,,	21·0	30·7	31·5	12·9	2·4	0·0	46·0
90 ,,	13·7	22·8	24·0	7·9	1·9	0·0	44·6
Total N after 90 days, mgm. per 100 gm. of sand*	49·1	77·0	59·7	36·1	32·5	—	98·2
Organic N after 90 days (Total N — NH_4-N), mgm. per 100 gm. of sand	35·4	54·2	35·7	28·2	30·6	—	53·6
Organic C after 90 days (%)*	0·248	0·315	0·152	0·273	0·382	0·449	0·530
C : N ratio (organic) after 90 days	7·1 : 1	5·8 : 1	4·3 : 1	9·7 : 1	12·7 : 1	—	10·1 : 1
Total N added, mgm. per 100 gm. of sand	93·6	89·0	89·2	69·2	45·2	6·6	106·8
% of added N mineralised	62·2	39·1	60·0	59·3	32·3	(0)	50·8
α-humus (%)	None	0·24	0·03	0·08	0·15	0·24	0·22
% N in α-humus	—	7·9	8·8	5·1	3·2	0·7	3·6

* Contents of N and C in a blind experiment (sand without addition, after 90 days), amounting to 1·6 mgm. of N and 25·0 mgm. of C, subtracted.

† 2 per cent. of each material.

organic N at end of experiment) was somewhat higher than in the soil, where, however, the measurement of nitrification was based on the excess of NO_3-N over control soil.

The α-humus determinations showed that several substances had left varying amounts of α-humus-like materials; this was true not only of *Mycogone*, *Stachybotrys* and *Polyporus* (which from previous experiments are known to yield such substances), but also of *Asp. fumigatus*, *Asp. carbonarius*, *Act. griseus*, and even the extracted mycelium of *Asp. fumigatus*. In the last instance the humus-like materials were undoubtedly synthetised during the process of decomposition, since all alkali-soluble matter had been removed from the mycelium, but in the other cases it was apparently present in the original mycelium. The fresh materials of *Asp. fumigatus*, *Asp. carbonarius*[1], *Mycogone*, *Stachybotrys*, and *Polyporus* yielded by extraction with hot NaOH solution a deep brown to black extract which, on acidification, gave a voluminous, flocculent precipitate of α-humus-like appearance. In the other organisms the presence of such a material could not be ascertained; if present at all, it was masked by the protein precipitated by the acid, and thus could have been there in small quantities only. The percentage of N in the α-humus was in most cases rather high—up to 8·8 per cent.—but *Mycogone*, *Stachybotrys*, lucerne and lupin yielded a material of nearly the same N content as soil α-humus. The straw gave, of course, a very N-poor humus (probably nearly pure lignic acid), but the mixture of straw and *Zygorhynchus* mycelium showed an interesting result; the amount of α-humus was not higher than with straw alone, but its N content was the same as that of soil α-humus. *Stachybotrys* was the fungus which yielded most α-humus; sufficient material was available to make a carbon determination, which showed a content of 49·6 per cent. C, a value slightly lower than that of soil α-humus (52–55 per cent.).

Enough material was available from *Polyporus* to obtain a preparation of the humus-like fraction of the fresh material and carry out a decomposition experiment with this. The fungus material was extracted with boiling 4 per cent. NaOH, the black filtrate acidified with sulphuric acid, of which enough was added to give an excess of approximately 2 per cent., and boiled for 30 minutes in order to hydrolyse the large amount of pectic material, which the alkali had dissolved. The dark brown amorphous residue was washed free from acid and dried. It contained in air-dry condition (with about 95 per cent. dry matter)

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[1] In the case of this organism the black matter seems confined to the spores; it is probably identical with the alkali-soluble black pigment *aspergillin* studied by Linossier (27) and supposed by him to be related to hematin.

5·08 per cent. N, 55·4 per cent. C, and only a trace of ash. Its decomposability was tested in garden soil (control soil II of the previous section) and in sand, where it was also compared with soil α-humus, containing 3·97 per cent. N and 52·2 per cent. C. The materials were added in quantities of 1 per cent., and kept at 25° C. for 180 and 240 days, respectively. The results are seen from Table IV.

Table IV. *Decomposition of humus from* Polyporus, *compared with soil humus.*

	Experiment in sand		Experiment in soil	
NO_3-N, mgm. per 100 gm. of soil	Sand + humus from soil	Sand + *Polyporus* humus	Control soil	Soil + *Polyporus* humus
At start	0·0	0·0	0·6	0·6
After 60 days	0·0	Trace	4·2	0·7
„ 90 „	Trace	0·0	6·8	2·9
„ 120 „	2·7	Trace	11·5	6·0
„ 180 „	4·7	3·9	7·8	8·5
„ 240 „	5·0	5·6	—	—
% of added N nitrified	12·8	11·0	—	(1·4)
% C at end of exp.	0·44	0·34	—	—
Final C : N ratio	12·7 : 1	7·0 : 1	—	—
% α-humus at end of exp.	—	0·32	1·33	1·90
% N in α-humus	—	8·3	4·1	5·0

The *Polyporus* humus proved to be a very resistant material: there was a slight, perhaps insignificant, nitrate formation from it in the soil after 180 days, and this was preceded by a reduction in nitrate content (in comparison with control soil). The humus determination showed that a considerable part of the added material, though not all of it, was recovered after 180 days, and there was a marked increase in the N content of the α-humus. This suggested that the non-nitrogenous parts of the *Polyporus* humus underwent decomposition, but that the nitrogenous constituents were left largely intact. In the sand experiment the *Polyporus* humus proved quite as resistant as the soil humus, but in both cases there was quite a considerable nitrification after 180–240 days. No appreciable amounts of ammonia were found here or in the soil experiment. Again, the carbon compounds were attacked to a considerable extent, so that the final C:N ratio was narrowed.

Also the N content of the α-humus at the end of the experiment was much higher than at the start, and equal to that of the α-humus from decomposition of *Polyporus* shown in Table III. This seemed to indicate that the humus-like fraction of *Polyporus* consists of a core of some highly resistant material rich in nitrogen, probably a protein, combined with some more easily decomposed non-nitrogenous matter.

Polyporus is essentially a wood-destroying fungus and as such is unlikely to be of importance in field soil, but in forest soils where it is growing, it cannot fail to contribute to the formation of nitrogenous soil humus, on account of the marked resistance shown by the humus-like fraction which it contains. Since this is soluble in alkalies, precipitated by acids and insoluble in alcohol, it must be found in preparations of "humic acid" from such soils. Undoubtedly other higher fungi contain materials with similar properties. In the field soils, on the other hand, it was found (Jensen (22, 23)) that *Mycogone nigra* and *Stachybotrys* sp. are active in cellulose decomposition, while *Aspergillus fumigatus* is regularly active in manure, at least under aerobic conditions. The resistant, alkali-soluble humus-like N compounds contained in their mycelia must therefore be found in the α-humus fraction of the soil, possibly together with similar materials formed by other micro-organisms (*e.g. Actinomyces griseus*). In this manner, these fungi no doubt contribute to the formation of α-humus or "humic acid" in the soil. To this extent the theory of Waksman (42) is supported by the present work, but the formation of humic substances, at least in significant quantities, is not a property common to all kinds of microbial protoplasm. It is possible that these nitrogenous compounds are able to form adsorption compounds with lignin or its derivates, which may account for the impossibility of separating nitrogenous and non-nitrogenous humus (see Hobson (18)).

The amounts of α-humus found in these experiments were as a rule quite small, and accounted for only a small proportion of the untransformed N. All the microbial substances thus seem to contain more or less of highly resistant nitrogenous materials in addition to the α-humus fraction, which is not always present. A part of this nitrogenous residue is, of course, contained in the cells of the bacteria and other micro-organisms which have decomposed the dead microbial matter. In order to obtain an idea of the amounts of N that might be accounted for in this way, a control experiment was carried out with a material which represented about the same amounts of carbon and nitrogen as the microbial substances, but which was readily available as microbial food and did not itself contain any resistant material. For this purpose a mixture of saccharose and asparagine in varying proportions was used, as follows:

I. 3·0 gm. of saccharose + 1·0 gm. of asparagine
II. 3·6 „ + 0·4 „
III. 3·75 „ + 0·25 „
per 200 gm. of sand.

The experiment was arranged in the same manner as that in Table III, save that a mineral nutrient solution (0·1 per cent. K_2HPO_4, 0·05 per cent. $MgSO_4$, 0·05 per cent. NaCl) was substituted for distilled water. The results are shown in Table V.

Table V. *Decomposition of saccharose-asparagine mixtures in sand.*

	I	II	III
NH_4-N, mgm. per 100 gm. of sand	3 Sacc. + 1 Asp. (C : N = 8·9 : 1)	9 Sacc. + 1 Asp. (C : N = 22·1 : 1)	15 Sacc. + 1 Asp. (C : N = 35·6 : 1)
After 10 days	40·4	15·6	6·6
,, 20 ,,	48·8	19·5	9·7
,, 40 ,,	13·0	9·1	5·5
,, 60 ,,	6·4	6·4	2·5
,, 90 ,,	3·9	2·9	Trace
Total N after 90 days, mgm. per 100 gm. of sand	14·9	10·9	7·0
Organic N after 90 days (total N − NH_4-N), mgm. per 100 gm. of sand	11·0	8·0	7·0
Carbon after 90 days, mgm. per 100 gm. of sand	61·0	52·0	46·0
Final C : N ratio (organic)	5·6 : 1	6·5 : 1	6·6 : 1
Total N added, mgm. per 100 gm. of sand	88·5	37·4	23·3
% of added N mineralised	87·6	78·6	70·0
α-humus at end of exp.	None	None	None

Here, as in the experiment in Table III, no formation of nitrate or nitrite could be detected. At the end of the experiment there remained a residue of organic N, rather constant in comparison with the amounts of N supplied, but much lower than the corresponding amounts shown in Table III, where less readily available sources of energy were supplied. This renders it highly probable that the nitrogenous residues found in the experiments with dead microbial matter are not merely contained in the cells of the organisms that have carried out the decomposition, but that they consist also largely of highly resistant, alkali-insoluble N compounds from the decomposed microbial substance. It is possible that compounds of this kind constitute, or at least contribute to, the practically unknown fraction of soil organic matter which is generally referred to as "ulmin" or "humin." It does not appear likely that this matter consists of nucleic compounds, since these are soluble in alkali and precipitated by acid, but the question cannot be answered on the basis of the data available here.

(4) *Decomposition of chitin.*

The fact that one of the resistant nitrogenous fractions, which are present in the microbial substance, is insoluble in alkali, suggests the possibility that this fraction may be represented by the chitin, which is present in the cell walls of the fungi in appreciable quantities. Chitin is often referred to as a particularly resistant compound, and several authors (*e.g.* Müller (30) and Beijerinck (5)) have hinted at the possibility that it may contribute to soil humus. The idea of its resistance seems to have been derived from its marked insolubility in chemical reagents, which, however, does not seem to be correlated with a resistance to attack by microbial agents. Benecke (6) was the first to describe a bacterium capable of decomposing chitin from Crustacea, and Störmer (36) stated that *Actinomyces chromogenus* could decompose fungal chitin. Folpmers (14) confirmed the fact of the decomposability of chitin; he found actinomycetes especially active in the decomposition of chitin in the soil. Waksman (42) observed that the acid and alkali-insoluble fraction of fungal mycelium, which probably consisted largely of chitin, was decomposed fairly rapidly in sand or soil. The same is seen to be the case with the extraction residue of *Aspergillus fumigatus* in the present experiments (Table III).

In order to obtain some more definite information concerning the resistance of chitin to microbial attack, a decomposition experiment with chitin was carried out. Chitin was prepared in the following way: fresh fruit bodies of *Lepiota* sp. were dried, ground finely, and extracted first with boiling 2 per cent. H_2SO_4 for 30 minutes, then after filtering with 5 per cent. NaOH (1 litre to 100 gm. of material) at 100° C. for 30 minutes, after which the material was allowed to settle, the dark liquid was poured off and replaced by fresh NaOH and the process was repeated until the solution was almost colourless. The material was then suspended in water acidified with H_2SO_4, the residue was filtered off, washed, and suspended in dilute (about 2 per cent.) H_2SO_4, to which a solution of $KMnO_4$ was added, until the mixture remained pink. The mixture was left overnight and the residue filtered off and boiled with dilute HCl till all MnO_2 had been dissolved. The residue, consisting of chitin, was then filtered off, washed free from acid, dried and ground. About 11 gm. of chitin was obtained from 200 gm. of fungus material. It appeared as a very light grey mass still showing organised structure, and contained in air-dry condition 4·16 per cent. N, 38·2 per cent. C, and 18·8 per cent. ash as impurities. The decomposition experiment was carried out in two soils: (1) a heavy clay soil, poor in organic matter and of *p*H 6·2, from Hoos Field; and (2) garden soil from a previous experiment (Table II, Control IV). Soil portions of 250 gm. received additions of 1·0 per cent. chitin and were kept in a moist condition at 25° C. for 120 days. The resulting changes in the microflora are represented in Fig. 6. In the Hoos Field soil there was a very marked increase in the bacterial numbers after 20 days, but later the numbers fell and at the end of the experiment were as low as those of the control soil. The actinomycetes also multiplied greatly owing to the addition of chitin, and this effect persisted through the whole period of the experiment. In the garden soil the effect of the chitin on the bacterial numbers was comparatively slight and perhaps not even significant, but after 10 days there was a very strong development of actinomycetes (in agreement with the results of Störmer (36) and Folpmers (14)) which later subsided, so that the numbers fell gradually towards the end of the experiment. A count of fungi in the Hoos Field soil on the 20th day showed the following "numbers" of fungi:

Control soil: 130,000 per gm. of soil.
Soil + chitin: 590,000 ,,

In the soil with chitin, *Mycogone nigra* and a *Fusarium* were very prevalent, accounting for respectively 36 and 21 per cent. of the total numbers of fungal colonies. These two fungi, as well as two actinomycetes from the garden soil, were isolated, and grown on pure sand with chitin and mineral nutrient solution. They all proved capable of growing well on the chitin, and of producing ammonia from it.

Table VI. *Decomposition of chitin in Hoos Field soil and garden soil.*

	Hoos Field soil				Garden soil			
	Control		+Chitin		Control		+Chitin	
NO_3-N and NH_4-N, mgm. per 100 gm. of soil	NO_3-N	NH_4-N	NO_3-N	NH_4-N	NO_3-N	NH_4-N	NO_3-N	NH_4-N
At start	1·4	0·4	1·4	0·4	2·8	0·0	2·8	0·0
After 10 days	—	—	—	—	—	—	11·0	9·1
,, 20 ,,	5·0	0·0	3·3	11·2	—	—	21·8	0·0
,, 40 ,,	9·1	0·0	15·7	5·9	9·5	0·0	26·2	0·0
,, 60 ,,	—	—	24·6	Trace	—	—	29·4	0·0
,, 120 ,,	6·6	0·0	31·6	0·0	12·8	0·0	39·2	0·0
% of chitin N nitrified at end of exp.			60·1				63·5	

The chemical changes are represented in Table VI, which shows that the chitin in both soils was readily transformed into ammonia and nitrate, and the nitrification was decidedly more complete than that of the untreated microbial substances of a similar C:N ratio in Table II. It is thus evidently not to the chitin that we have to look for the resistant nitrogenous fraction of microbial substance.

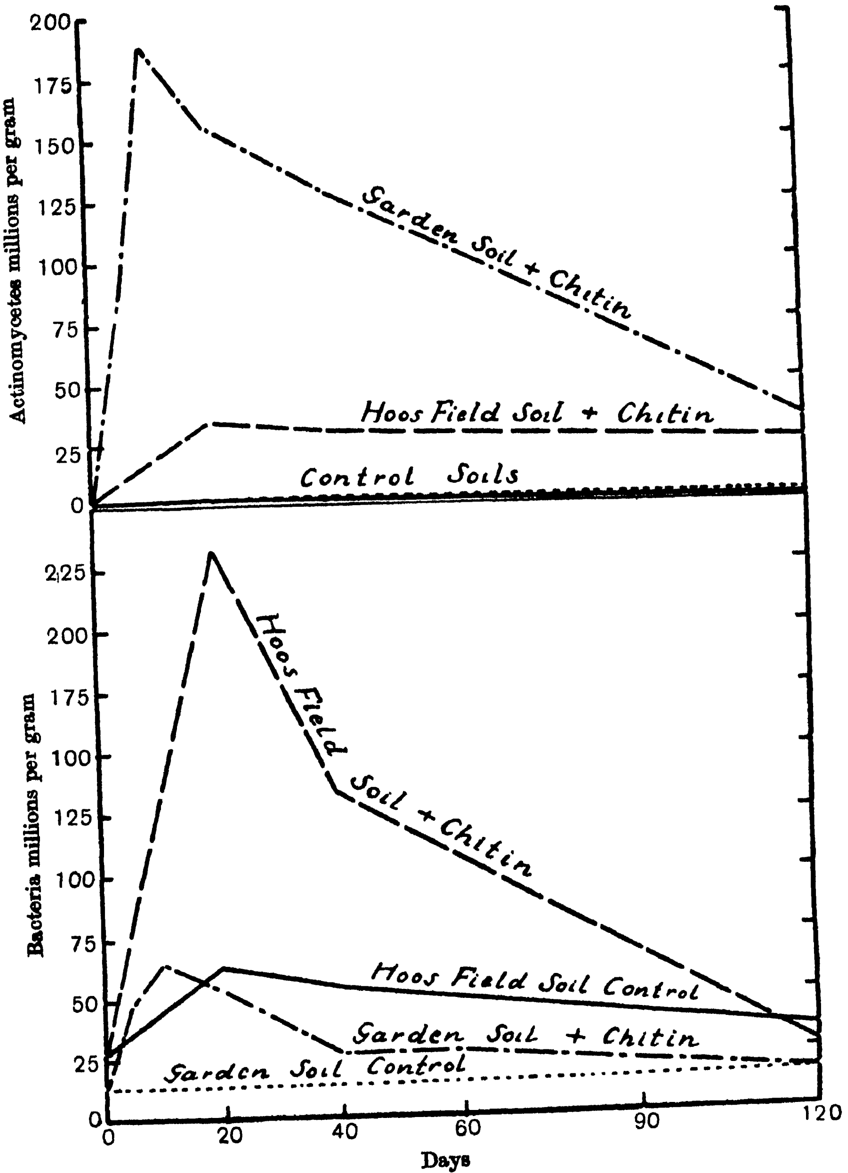

Fig. 6. Numbers of bacteria and actinomycetes in Hoos Field soil and garden soil and chitin.

GENERAL CONCLUSIONS.

The experiments showed that a certain portion of the N of microbial substance is very rapidly transformed into ammonia and nitrate, while the rest seems resistant to decomposition and is very slowly transformed. This remainder is composed of the following fractions:

(1) Nitrogen assimilated by the active micro-organisms.
(2) Nitrogen present in α-humus-like compounds (not always present).
(3) Nitrogen present in alkali-insoluble compounds perhaps comparable with the "ulmin" or "humin" of the soil.

The history of the nitrogen changes is set forth in Fig. 7. It must be borne in mind that we are dealing not with static, but with dynamic phenomena. The synthetised cell material, which represents fraction (1), undergoes a renewed decomposition, through which it again gives rise to a residue of fraction (3) and possibly (2). These compounds will in their turn undergo a slow decomposition, giving rise to new cell material, of which every generation leaves a residue of more resistant material.

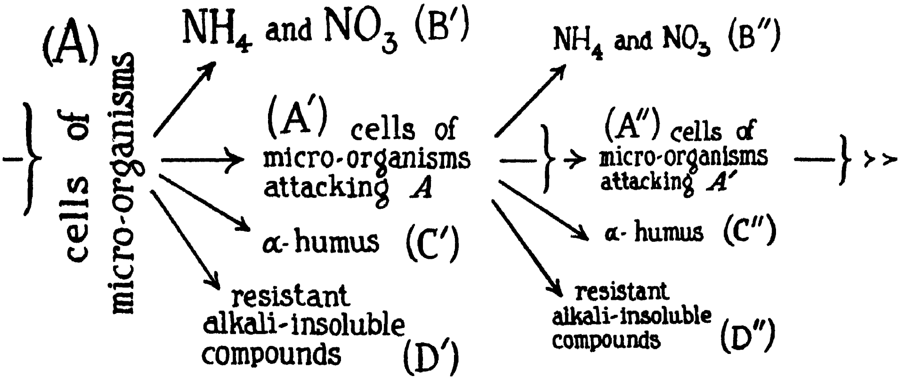
(A)
cells of
micro-organisms
NH_4 and NO_3 (B′)
(A′) cells of micro-organisms attacking *A*
α-humus (C′)
resistant alkali-insoluble compounds (D′)
NH_4 and NO_3 (B″)
(A″) cells of micro-organisms attacking *A′*
α-humus (C″)
resistant alkali-insoluble compounds (D″)

Fig. 7. The progressive repartitions of nitrogen derived from micro-organisms.

These phenomena here discussed, together with the results of previous experiments (Jensen (22, 23)), lead us to the following conception of the decomposition of manure in the soil:

A considerable part of the organic N of the manure (18–25 per cent.) is present from the beginning in compounds corresponding to the α-humus of the soil, which are in themselves very resistant to decomposition. Another part of the manure N is used for the synthesis of cells of bacteria and other micro-organisms, which multiply vigorously immediately after the addition of the manure to the soil, utilising the carbonaceous compounds of the manure as sources of energy and sometimes reaching total numbers so high that their cell substance may account for 25 per cent. of the amount of N added in the manure. If the amount of N available to the bacteria is insufficient in proportion to the amount of available energy, N is assimilated from the soil, and the accumulation of nitrate decreases in comparison with unmanured soil. Some of the assimilated N may be converted into resistant, α-humus-like compounds through the activity of cellulose-decomposing fungi, especially if fresh straw has been present in the manure. In addition, the micro-organisms in general seem to contain other resistant nitrogenous compounds which must also be present in the farmyard manure, since it is very rich in living and dead micro-organisms. The remaining N of the manure, if any, is rapidly converted into ammonia and nitrate. Later, fresh inorganic C is released when the multiplication of the bacteria stops and their cells undergo nitrification. The resistant compounds, which the micro-organisms have built up, tend to accumulate, together with the α-humus, and contribute to the soil humus. The C:N ratio of the manure exerts a marked influence upon its nitrification. A widening of this ratio, *i.e.* an increased supply of energy in proportion to nitrogen, tends to bring about a larger development of micro-organisms and thus to increase both the amount of N temporarily locked up in microbial protoplasm and the amount of N converted into resistant compounds.

Farmyard manure resembles many other organic nitrogenous materials in containing a fraction of the N in a state resistant to decomposition and a remainder that is readily nitrified. In the case of farmyard manure, however, the resistant fraction is particularly large.

SUMMARY.

1. Dried cell substance of various micro-organisms—nine fungi, one actinomycetes, two bacteria—were subjected to decomposition in soil and sand. In neutral garden soil, the addition of microbial substance gave rise to a more or less abundant, but always temporary, development of bacteria and actinomycetes; the latter group of organisms was often stimulated to a very conspicuous degree.

2. The development of micro-organisms was accompanied by a more or less abundant production of nitrate. After 60 days 19–61 per cent. of the added N had been nitrified, and after 120 days the proportion had in most cases not increased very materially.

3. A certain fraction of the N of microbial substance is thus very

readily nitrified, while the rest persists in the soil as an almost unnitrifiable residue. No clear influence of the C:N ratio of the materials was noticeable.

4. Experiments in sand showed an abundant ammonia production after 10 days. After 90 days 25–64 per cent. of the N was left as organic residue. Several fungus materials had left some matter of the character of α-humus: colloidal, brown to black compounds, soluble in alkali, precipitated by acid, and containing 3·6–8·8 per cent. N and 50–55 per cent. C. The mycelia containing these substances left more organic N behind than the others. Among the fungi producing them were *Mycogone nigra* and *Stachybotrys* sp., which have been found active in cellulose decomposition in soil. The humus-like material prepared from *Polyporus* sp. proved as resistant to microbial attack as did soil humus.

5. The amounts of organic N left in the sand experiment appeared larger than what would be expected, if these residues were only the remains of the organisms which had decomposed the added substances, plus the α-humus-like material where this was present. It seems that the microbial substance generally contains some material—apart from the α-humus fraction—which does not readily undergo decomposition. This substance is not identical with the fungal chitin, which was found to be readily nitrified in the soil and to give rise to an abundant development of actinomycetes.

The author feels it a pleasant duty to express his most sincere thanks to Sir John Russell, D.Sc., F.R.S., Director of the Rothamsted Experimental Station, for placing the facilities of the laboratories at his disposal, and to Dr H. G. Thornton, Head of the Department of Bacteriology, for his helpful interest in the work and for reading the manuscripts.

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LXIX. THE BIOLOGICAL DECOMPOSITION OF PLANT MATERIALS.

VII. THE NATURE OF THE RESIDUAL HEMICELLULOSES OF ROTTED STRAW.

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In the first paper of this series [Norman, 1929, 1] a detailed method was described for the evaluation of the furfuraldehyde-yielding constituents of plant materials, and the nature of these substances in straws was determined. Later, by employing these methods on decomposing straws, the loss of such constituents was followed and their rôle in decomposition investigated [Norman, 1929, 2].

The direct estimation of hemicelluloses has not yet been satisfactorily achieved owing to their heterogeneous structure, and accurate evidence of their removal as a whole was not possible. It was only possible to follow with certainty the changes in the content of pentose and uronic acid units. The hexose units present are indeterminable directly. Acid hydrolysis methods, such as have been proposed by Waksman and Stevens [1930], are unsatisfactory and erratic, inasmuch as it has been shown by Link and Niemann [1930] that the uronic acids are unstable in presence of the concentration of hydrolysing agent employed and undergo decarboxylation with the formation of reducing substances of unknown constitution.

As far as it was possible to judge from the loss of the pentose units in the hemicelluloses, these substances undergo a very rapid and extensive early loss in the decomposition of such materials as oat or rye straw. It seemed that about 50 % of the total hemicelluloses was fermented away in the first week, but that after this point, while the cellulose was being rapidly removed, the hemicelluloses suffered only a small but steady additional loss up to the end of the two months, which was the period of the experiment. Analyses of well-rotted materials which have been made at other times have indicated that when decomposition is practically at a standstill only traces of hemicelluloses remain.

The hemicelluloses are a very indefinite class of polysaccharides, and their physical properties are such that no separation is entirely satisfactory. Hydrolysis studies suggest that the type of linkage involved is similar in different fractions, and in hemicelluloses from different sources. Biological availability, however, does not depend only on linkage, but also on the nature of the units involved. The following sugars are commonly found—arabinose, xylose, glucose, galactose and mannose, together with glycuronic and galacturonic acids. These vary very much in their utilisation by micro-organisms, so that there is the possibility that certain groups may be preferentially removed, leaving intermediate residues which are attacked more slowly. In the studies already described, the losses of uronic acid from the hemicelluloses of oat straw were very different from those of the pentose units, while in the rye straw series they were rather similar. Attention was not drawn to this at the time. In view of the variations in availability between the constituent units of hemicelluloses it was thought possible that resistant groupings might remain, even at the close of an active fermentation, and an investigation was accordingly made of the nature and amount of the residual hemicellulose material of oat straw rotted under optimum conditions for many months.

EXPERIMENTAL.

One kg. of dry oat straw, well chaffed, was thoroughly moistened by spraying and placed in a large bottle to which available nitrogen in the form of ammonium carbonate was added at the rate of 1·0 g. N to 100 g. straw. The bottle was incubated at 35° and frequently turned to secure even distribution of the nitrogen and thorough and uniform wetting. From time to time additional water was added from a spray, water-logging, however, being avoided. Rapid decomposition set in, and the straw shrank in bulk and darkened in colour. By the end of nine months the straw had rotted to a black pulpy mass, without cellular structure. A portion was taken for analysis and the remainder extracted with 1500 cc. of water on a water-bath for 4 hours. The residue after filtration was re-extracted twice with 1000 cc. water for the same period. The combined filtrates were concentrated under reduced pressure and poured into three volumes of alcohol, yielding a light brown precipitate (precipitate I). The dark residue was then extracted with 1500 cc. of alcoholic sodium hydroxide (2 % NaOH in 60 % C_2H_5OH) for 4 hours. Two further treatments with alcoholic sodium hydroxide followed. The very dark filtrates obtained were combined, nearly neutralised with acetic acid, and concentrated under reduced pressure. When of small volume acetic acid was added, and a dark heavy precipitate was obtained (precipitate II). The residual straw was then treated twice with 500 cc. 2 % NaOH for 4 hours at 80°, followed by 4 % NaOH at 100° for 4 hours. The residue was washed many times with hot water, and dried. The combined alkaline extracts were made definitely acid with glacial acetic acid, whereupon a precipitate, grey-brown in colour, was obtained and was centrifuged out (precipitate III). To the acid-aqueous residue were added 2½ volumes of alcohol, and the precipitate obtained was filtered off on fluted filter-papers (precipitate IV).

Purification of the fractions. Precipitate I was redissolved in water and reprecipitated first by acid alcohol and then by neutral alcohol, finally being dried in increasing concentrations of alcohol, and over P_2O_5 in a vacuum desiccator; yield $< 0 \cdot 5$ g. Precipitate II was washed several times with warm water and ether, and dried in a vacuum-oven; yield 8 g. Both precipitates III and IV were redissolved in alkali and precipitated by the addition of acid alcohol, finally being washed with alcohol and dried with increasing concentrations; yields, precipitate III $< 1 \cdot 0$ g.; precipitate IV $< 0 \cdot 5$ g.

The composition of the fractions. Owing to the smallness of the quantities available, the number of estimations which could be made was of course very limited. The pentose and uronic acid contents of the fractions were pre-eminently of interest and were determined in each case. Tests were made for mucic acid after oxidation with nitric acid, and, in the case of precipitate III, for arabinose by heating with diphenylhydrazine, in which case the result was positive.

The following results were obtained.

Precipitate	Ash %	Furfuraldehyde yield %	CO_2 yield %	Mucic acid
I	*	5·94	8·33	–
II	*	–	Trace	–
III	1·74	17·08	3·73	+
IV	*	12·38	3·12	+

* Not determined.

DISCUSSION.

The water-soluble precipitate I from the rotted material bore no obvious relationship to any original constituent of the straw. The fact that on oxidation it yielded no trace of mucic acid precludes any possibility of relationship to pectin. It seems probable that this substance represents a microbial product akin to the bacterial gums. Hopkins, Peterson and Fred [1930, 1931] have recently reported that the water-soluble gum produced by strains of *Rhizobium* is a polysaccharide consisting of glucose and glycuronic acid. An osazone prepared from a very small quantity of the hydrolysed precipitate I resembled glucosazone; but the quantity was insufficient for characterisation and too much stress is not put on this point. The analytical figures indicate the presence of 33·3 % uronic acid anhydride, which accounts for 5·55 % furfuraldehyde. The balance of the furfuraldehyde, 0·4 %, might be due to pentose groupings or more probably to experimental error. It seems therefore that this substance contains about 66 % hexosan and 33 % uronic acid anhydride.

Precipitate II was found to yield no furfuraldehyde or CO_2 on treatment with HCl. Further it did not give reducing sugars on hydrolysis with dilute acid. This fraction probably contains lignin and "humic" matter, possibly the "hymatomelanic acid" described by Odén [1919]. It was not investigated further since it appeared not to be of polysaccharide nature.

Precipitates III and IV may be considered together. They are the fractions prepared in the same way as the A and B hemicellulose fractions of the straw, and may be compared with them.

	CO_2 yield %	Uronic acid anhydride %	Total furfuraldehyde %	Pentose furfuraldehyde %	Anhydro-pentose calculated as anhydro-arabinose %	Anhydro-hexose by difference %
Precipitate III	3·8	15·2	17·4	14·9	27·6	57·2
Hemicellulose A	2·7	10·8	42·9	41·3	76·7	12·5
Precipitate IV	3·1	12·4	12·4	10·3	19·2	68·4
Hemicellulose B	7·9	31·8	43·3	38·0	68·0	—

The calculations of anhydro-hexose are made with some reserve, inasmuch as there is little evidence of the purity of fractions obtained in such small quantities. It is possible that they might be contaminated with substances of a humic nature, possessing similar properties, though it is likely that soluble substances of this class would be removed by the pre-treatment with alkali-alcohol. From the positive mucic acid tests the presence of galactose or galacturonic acid in both fractions may be inferred. In both cases the residual products were much lower in pentose content than the straw products. It is probably safe to conclude that these substances represent small unavailable fragments of the original hemicelluloses, rather than elaborated products, since no water-insoluble microbial constituents or products which contain uronic acids and mixed sugars are known. This investigation failed to reveal the presence of any considerable amount of biologically unavailable hemicellulose, or of any resistant grouping formed as a consequence of incomplete attack. Instead it emphasised the complete availability of the hemicelluloses, an availability quite remarkable. The kg. of dry straw originally contained about 230 g. of free hemicelluloses, of which, at the close of the fermentation less than 2 g. could be recovered. It provides clear evidence that the structural arrangement and architecture of the hemicelluloses in the cell-wall is not such that the attack of micro-organisms is in any way hindered or retarded, as it is in the case of cellulose, by the presence of a more resistant lignin barrier. The cellulose in the same period suffered a loss of only two-thirds.

Variations in the biological availability of the hemicelluloses, or of units or constituent groupings in them, cannot therefore be of much importance in the case of the decomposition of straws by a general mixed flora, though it is likely that in pure culture decompositions they would be more obvious. In the earlier stages of decomposition by a mixed flora it is possible that such differences might be detectable. Information on this point can only be obtained by the laborious method of preparation of the hemicellulose fractions at different periods during the decomposition.

SUMMARY.

1. The nature of the residual hemicelluloses of well-rotted straw has been investigated with a view to obtaining information on possible resistant groupings.

2. Only very small quantities were obtained, and there was no indication of variation in availability or the accumulation of less available groupings. The complete availability of these substances to biological attack is emphasised.

3. The results indicate that the distribution and arrangement of the hemicelluloses in the cell-wall are such that microbial attack is not hindered by the presence of any resistant barrier.

4. A water-soluble polysaccharide, probably of microbial origin, was also prepared. It contained 33 % uronic acid anhydride, and 66 % hexosan, and gave evidence of the presence of glucose units.

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ON *ATOMARIA LINEARIS* STEPHENS (COLEOPTERA, CRYPTOPHAGIDAE) AND ITS LARVAL STAGES

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(With 4 Text-figures.)

I. Introduction.

Atomaria linearis Stephens has been recognised for many years as an occasionally serious pest of mangolds. Curtis (1860) notices a record made by M. Bazin in 1839 and a few years later one by Macquart, who stated that fields of red beet near Lille were completely destroyed. There does not appear to exist in the literature, however, any account of its developmental stages, nor are its breeding habits definitely known. On account of the increased difficulty of finding the adult beetles around the young mangold plants as the season advances, *i.e.* toward the end of June, the assumption has been made that they migrate to other plants and perhaps breed there. The present writer has obtained the larval stages of the beetle by placing imagines upon growing mangold plants kept in the laboratory. The object of this article is to place on record a description of the larva so that it may be recognised in the field. It is hoped to deal more fully with the bionomics of *Atomaria* in a later paper.

II. General.

Miss Ormerod, who apparently gave the name "pigmy mangold beetle" to this insect, states in her report for 1892 that for some years previously enquiries had been sent to her regarding the nature of a very injurious attack on young mangolds. She had not at that time discovered the cause, but in that year Prof. Harker, of the Royal Agricultural College, Cirencester, communicated to her that he had, the year before, identified similar damage to mangolds with attacks by *Atomaria linearis*. He, therefore, appears to have made the first economic record of this beetle for England. That it was a well-known pest at this time on the Continent is evident from various accounts, *e.g.* that of Henze (1861) and Ritzema Bos (1891). Miss Ormerod gives further accounts of the beetle in her reports for 1895 and 1898 and suggests measures for control, two of which are thick seeding and rotation—as they are to-day. Although a notable feature of all the attacks mentioned is the enormous numbers of beetles present, she remarked in 1898 that "up to the present date, the attack has been so rarely observed in this country that it does not appear of much practical importance beyond pointing out to us to be ready to meet it if it should occur to a greater extent." Further outbreaks were noticed by Theobald in 1904, 1906, 1908, 1910, 1911, etc., the counties involved being Kent, Devon, Buckingham, Huntingdon, Somerset and Shropshire. With the introduction of the sugar-beet industry the beetle has found a new host, and is now frequently recorded in the monthly reports of the Ministry of Agriculture. No doubt its numbers have been considerably increased by continuous cropping with mangolds or sugar-beet, and one writer, in Czechoslovakia, refers to a recent attack as being the most severe in 20 years, as a result of a sequence of sugar-beet crops.

It will be noticed that Theobald's records are all from southern or western England. This would not seem to be entirely due to the location of this observer, since Fowler (1912) gives no record north of the Manchester district. On the Continent the beetle is a fairly frequent pest of sugar-beet, Rambousek (1926) in Czechoslovakia stating that 80 per cent. of the damage ascribed to Elaterids is due to its depredations.

III. NATURE OF ATTACK AND HABITS OF THE BEETLE.

The most serious damage to the mangold plant is caused by the adult beetle biting the "shoots" of the seeds as they germinate. Later on, the small tap root is pitted with characteristic holes which later turn black and, in bad cases, it is eaten completely through. Often, however, though completely ringed, the central tissues may be left intact, so that outwardly the plants appear healthy and not retarded. Such plants are easily broken off. Small irregular holes are often eaten in the leaves, but this damage is usually quite insignificant.

As the plant increases in size, the root attack becomes less dangerous, provided that good growing weather prevails. Beetles may be found at the same time both in the crown of the plant and in the surface layer of soil. When disturbed they feign death, and may be easily overlooked, unless given time to resume their activities. As was mentioned by Dowling (1908) the beetles were not found to be more active at night. This writer also refers to the apparent disappearance of the beetles, as the mangolds grow bigger, and states that by mid-June hardly a beetle could be found. He often noted them about this time or a little later, flying in large numbers in clearings in a wood some ⅛ mile from the nearest mangolds, and suggested that a migration had taken place.

In 1930 this apparent early disappearance was observed by the present writer also, but during 1931 an observation was made that may account for it in another way. In a plot of sugar-beet, a few beetles per plant could be collected early in the season by examining a number of consecutive plants. Later, at the end of June, a similar procedure produced no beetles at all—unless one was fortunate enough to find an individual plant which, for some unknown reason, proved to be specially attractive. In such cases the surrounding soil suggested the activities of a small ants' nest. It was swarming with beetles and numbers up to a hundred were counted. It is thought that the beetles may congregate at this period in numbers about individual plants—perhaps for breeding purposes—leaving the other plants free. Theobald reported that the numbers of beetles decreased about the end of July, in one year as late as the middle of August.

With regard to the amount of damage the beetles are capable of doing, it was found in the laboratory that a single one will prevent the development of a seedling that has just germinated, whereas, when the full cotyledon stage has been attained, the depredations of many did not noticeably affect it.

In addition to the cultivated mangold and sugar-beet, the observations of Lägenbuch and Schewket (1931) indicate that *Atomaria linearis* has other food plants in spinach, radish, marjoram, *Chenopodium album*, *Stellaria media* and *Polygonum aviculare*.

IV. REVIEW OF CONTROL MEASURES.

Theobald claimed good results from cross ring-rolling and so consolidating the soil around the seedlings. In this connection an observation of Dowling is of interest. He noted that, in a field severely attacked by *Atomaria*, a small area stood out as noticeably healthy. On inquiry it was found that a mistake had been made—rolling had been begun while the field was still too wet. Some amount of panning had therefore occurred and the beetles could not get to the plants. A similar effect of panning due to flooding was observed on an area of Barnfield on the Rothamsted Farm in 1931, but, in this case, the panning was so complete that the plants themselves were badly retarded. As the worst aspect of the damage is the early attack in the germinating stage, sowing deterrents with the seed is an obvious measure, and many Continental writers claim good results from this procedure. For example, the use of 1 lb. of naphthalene to 20 lb. of seed has been advised. In this country, in a recent report of research work (1931), the use of carbolic acid 1 per cent. and magnesium sulphate 5 per cent. is advocated as a seed steep, but the advantage of using the latter ingredient is by no means evident.

Lägenbuch and Schewket (1931), however, did not find that naphthalene treatment was of any value and state that spraying with nicotine is the only measure of use. Another spray fluid said to be effective, in Czechoslovakia, is a 1 per cent. solution of sodium or potassium cyanide. Rambousek (1926) suggests the trapping of the beetles in early autumn by means of holes in the ground suitably baited with sugar beet. In Denmark thick sowing and late thinning out is advised as a suitable measure and, from the observations made by the present writer during 1930–31, this would seem quite effective, though the attacks observed were not heavy.

V. General remarks on the life history.

It seems to be established that *A. linearis* hibernates as an adult in soil (Morris, 1927) or among debris. Dowling records it as attacking April-sown mangolds. It first came under observation by the writer in late May attacking mangolds, and in early June on sugar beet. Copulation was to be seen occurring in early June, and couples were frequently taken throughout the month and during the first week of July. It was observed to last for over 2 hours in some cases and was seen to take place only in the soil. Theobald (*loc. cit.*), however, notes pairing as occurring on the wing. As already stated the beetles were difficult to find after June, and in the laboratory they began to die off at the beginning of August. Rambousek refers to finding enormous numbers in early autumn in debris of beet fields, and presumably this is the new generation. There would, therefore, appear to be only one brood in the year.

Laboratory observations.

It is difficult to induce *Atomaria* to oviposit in captivity, a fact already noted by Theobald and by Dowling who both failed to obtain eggs. A number of devices were tried in order to facilitate the search for the necessarily minute eggs and young larvae. Moist black filter paper was used instead of soil; plants were grown in tubes of water-culture solution, allowing the beetles access to the upper regions of the plant only, by passing it through a cotton-wool-plugged hole in a cork, the lower part of the root system being immersed in the solution; other plants were placed in tubes embedded in a block of plaster of Paris that was kept moist in a bath of water-culture solution; still others in open-ended tubes, plugged with cotton-wool and containing a minimum quantity of soil, the whole being sunk into a pot of soil. A few eggs were obtained by these means and one or two were recovered by washing small quantities of infected soil. It was found, however, that normally grown plants were best for later stages, and the larvae were recovered from them by suspending the soil on wire gauze over water. Both mangold and sugar beet were used as host plants, but actually the larvae were recovered from mangold plants.

The eggs took from 4 to 6 days to hatch under laboratory conditions. From a pot set up out of doors in the middle of June, half-grown larvae were recovered during mid-July and fully grown larvae early in August. The egg and larval stages occupy, therefore, at a maximum, a period of 6 weeks.

The larvae apparently live freely in the root system of the plants. Occasionally the Malpighian tubules are red in colour, resulting, it is believed, from the ingestion of red pigment of the epidermal layers of the upper part of the tap root of sugar beet.

Description of the egg and larval stages.

Few Cryptophagid larvae have been hitherto described and only one of these belongs to the genus *Atomaria*. The species concerned is *A. nigripennis* Payk., of which an inadequate account is given by Erichson (1848). The larva of *A. linearis* is, therefore, described in some detail.

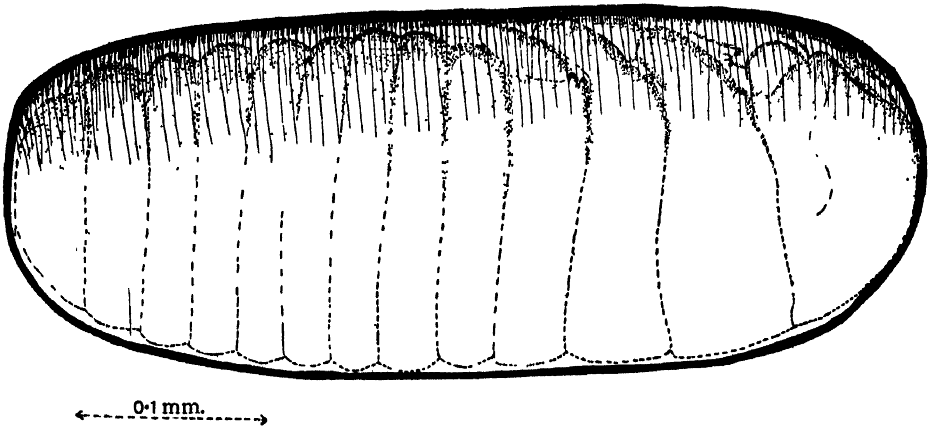

Fig. 1. The egg with developing larva inside.

The egg (Fig. 1) is elongate-oval, measuring just under ½ mm. long

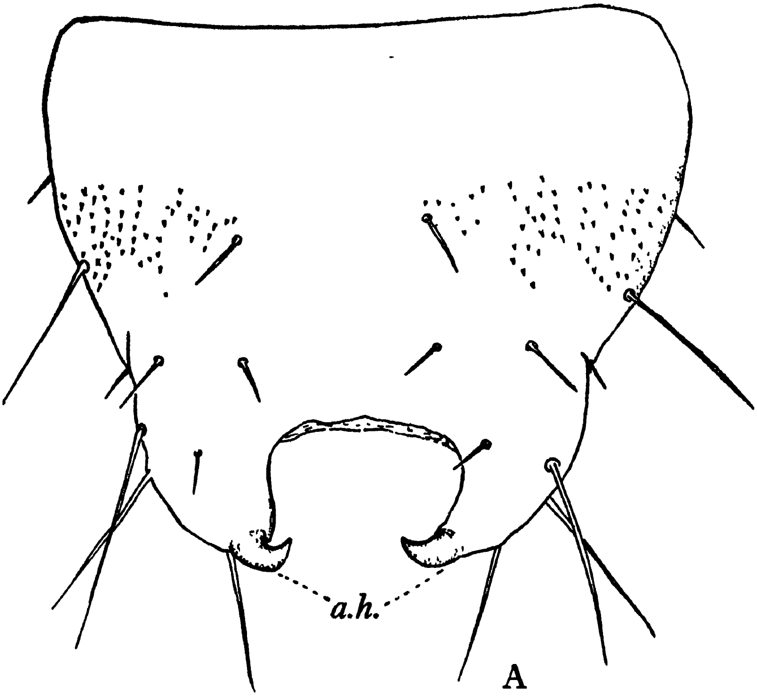

Fig. 2. A, last abdominal segment of fully grown larva from above. *a.h.*, anal horns. × 300.

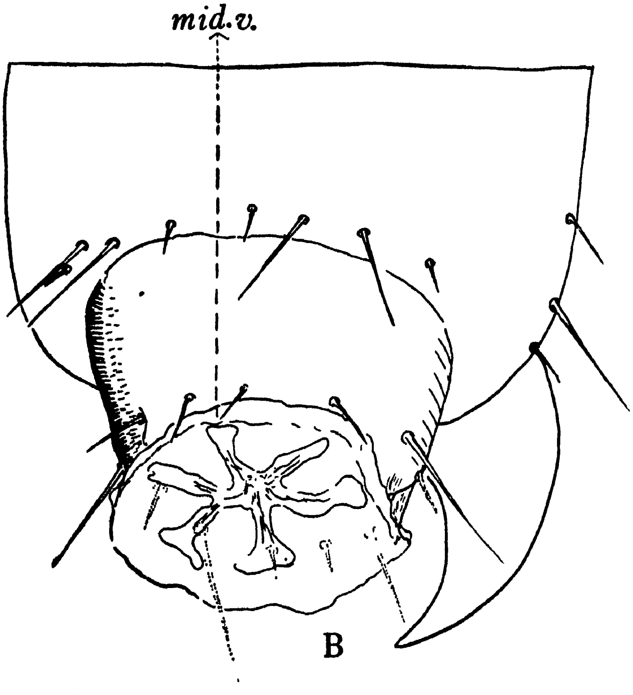

Fig. 2. B, from beneath, slightly from one side.

and about 0·18 mm. in width. The colour is translucent greyish and there is no sculpturing.

The newly hatched larva is about ½ mm. long and 0·15 mm. across. It differs from later stages in that, relative to body size, the head, spiracles, claws, and anal horns are very much larger. The setae also are proportionately much larger and give the larvae a spiny appearance which is lost later on. The arrangement of the setae is, however, essentially similar in all stages and is described for the fully grown larva.

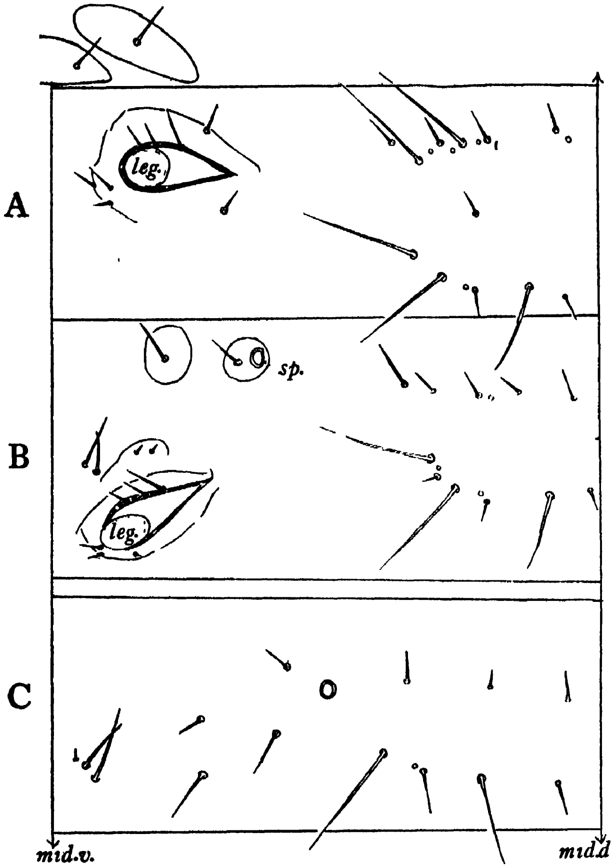

Fig. 3. Map of setal arrangement on A, prothorax; B, mesothorax; C, abdominal segments 1–8. *leg.*, section of insertion of leg; *mid.v.*, mid-ventral; *mid.d.*, mid-dorsal line. *sp.*, spiracle.

The fully grown larva (Fig. 4 A) is just under 3 mm. long, some 0·4 mm. across, and of a translucent greyish colour. Sclerotisation is weak, the head capsule and anal horns being scarcely coloured. The three thoracic segments bear three pairs of weakly chitinised legs (Fig. 4 G),

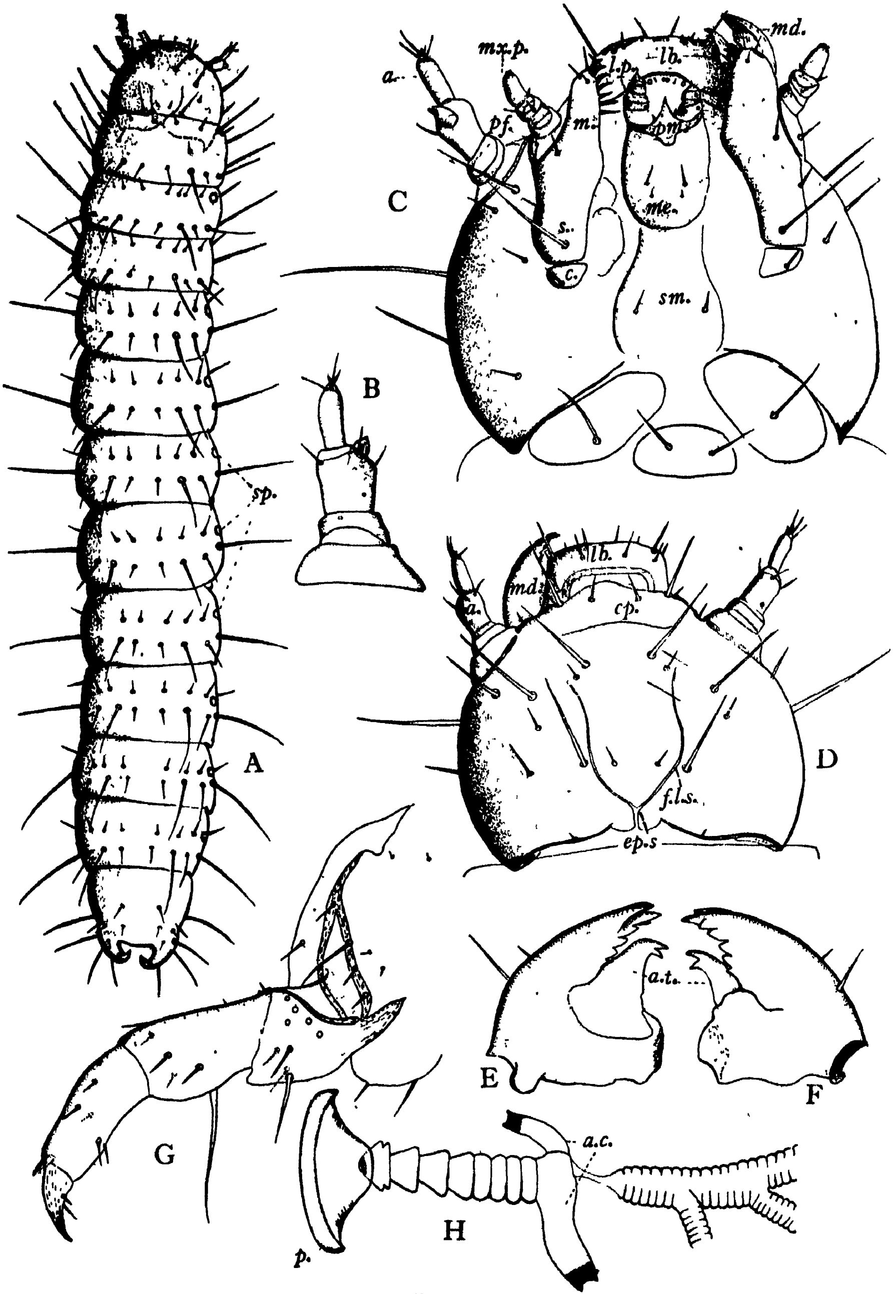

Fig. 4.

well armed with spines and ending in a strong terminal claw. There are nine abdominal segments of which the last is narrowest. This carries dorsally two inwardly and upwardly directed, curved, seta-bearing horns and ventrally, a papilla carrying the anus. This is presumably the reduced tenth segment judging from its setae. The mesothorax and first eight abdominal segments each bear a pair of spiracles.

The arrangement of the setae on the prothorax differs from that on the last two thoracic segments. On the abdomen the setal arrangement is alike on the first eight segments but that on the ninth segment (Fig. 2) exhibits certain differences.

A chart showing the disposition of the setae on the prothorax, mesothorax and abdominal segments 1–8 is given in Fig. 3.

It will be noticed that between head and prothorax in the ventral position there is a median unpaired plate and two lateral plates which may correspond to the spiracular and its accompanying plate shown in the other thoracic segments. Each of the body segments shows a median dorsal suture.

The head (Fig. 4 C and D) is approximately circular in outline and somewhat flattened, with the epicranial and fronto-lateral sutures well-developed posteriorly, while indications of ante-clypeal and post-clypeal sutures are present in front. The setal arrangement is shown in the figures. The antenna (Fig. 4 B and *a.*, Fig. 4 C and D), has three joints excluding the base; the second segment is the largest and carries ventrally and externally a small conical lobe as well as the elongate last segment. The antenna thus has a biramous appearance. The last two segments carry three spines and two peg-like structures.

The labrum (*lb.*, Fig. 4 D) bears two transverse rows of setae and the anterior margin is armed with short spines. The mandibles (Fig. 4 E

Fig. 4.

A. Dorsal view of larva, about half-grown. × 60.
B. Dorsal view of right antenna. × 250.
C. Ventral view of head capsule of fully grown larva, with right mandible removed. × 180.
D. Dorsal view of same. × 140.
E. The right mandible ventral aspect. × 260.
F. The same, dorsal aspect.
G. Right metathoracic leg, inner aspect. × 250.
H. Spiracle from abdominal segment in side view. × 900.

a. antenna, *a.c.* arms of closing apparatus, *a.t.* auxiliary tooth, *c.* cardo, *cp.* clypeus, *ep.s.* epicranial suture, *f.l.s.* fronto-lateral suture, *lb.* labrum, *l.p.* labial palp, *m.* mala, *md.* mandible, *me.* mentum, *mx.p.* maxillary palp, *p.* peritreme, *pf.* palpifer, *pm.* prementum, *s.* stipes, *sm.* submentum, *sp.* spiracles.

and F) have two main teeth, the dorsal inner surface of the upper tooth having serrations or smaller teeth. In addition there is an auxiliary tooth (*a.t.*) arising from the inner side of the molar area and ending in two strong hooked denticles.

The maxilla consists of a single lobe, the mala (*m.*), and a reduced maxillary palp (*mx.p.*). The palp consists of three segments and a basal area, the palpifer (*pf.*). The penultimate segment carries two small spines and the apex of the last segment is beset with a group of papillae; on the dorsal aspect of the last segment is a long peg-like structure. Cardo (*c.*) and stipes (*s.*) are present, the first-mentioned sclerite being small.

The labium is considerably reduced, consisting of two-jointed peg-like palps (*l.p.*), a prementum (*pm.*) and only indications of mentum and submentum (*m.* and *sm.*).

The tracheal system presents no features of special interest. There are present the usual mesothoracic and eight abdominal pairs of spiracles. All are of similar structure, the mesothoracic spiracle differing only in its slightly larger size and its more ventral position. The peritreme (*p.*, Fig. 4 H) is circular and surrounds a cup-shaped depression, at the bottom of which is a slight protuberance. This carries the actual opening of the spiracle and widens out into a short tube provided at its base with two projecting arms, one longer and one shorter (*a.c.*, Fig. 4 H). These are part of the closing mechanism and serve for the attachment of the muscles concerned.

Below the arms is a constriction which opens out into the main tracheal tube.

6. SUMMARY.

1. A brief survey is made of the history and habits of *Atomaria linearis* Stephens, the Pigmy Mangold Beetle, and some new observations recorded.

2. The egg and the external structure of the larva are described for the first time.

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STUDIES OF FLUCTUATIONS IN INSECT POPULATIONS

I. THE INFESTATION OF BROADBALK WHEAT BY THE WHEAT BLOSSOM MIDGES (CECIDOMYIDAE)

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(*With six Figures in the Text.*)

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I. Foreword.

The number of insects in any population varies from time to time on account of many factors, such as disease, lack of food, climatic conditions and the intervention of natural enemies and man. The fundamental and economic importance of any additional information regarding such fluctuations is very obvious, especially in the case of insects not easily controlled by the usual methods. For this reason, the gall midges (Cecidomyidae) are pre-eminently suitable for such a study, being also readily obtainable in large numbers.

There are several means of approaching the subject. One favourite method is to keep a population under controlled conditions, whether constant or fluctuating, in the laboratory and to determine the effects on the insects in question. Another method is to make field observations of a phenological type and then attempt to correlate noticed fluctuations with the factors involved. There are obvious weaknesses in employing either of these two methods by itself. Two such drawbacks may be mentioned. It is very easy to use conditions in the laboratory that would not occur in a state of nature; and phenological observations depend too largely on the personal factor. The technique necessary to avoid the latter renders the method too extravagant in man-power for general use.

The method adopted by the author has been to take samples of wild populations in the fully fed larval stage and to rear, to the imaginal stage in an outdoor insectary, both the host insect and its parasites. Care has been taken to check results by plentiful observations in the field. Certain procedures, which are independent so far as is possible of the individual worker, have been followed, in order that the study could be put on a routine basis if necessary. In this way it would be possible for the study to be carried on over a longer period of years than if only one worker were involved.

This study of insect populations is designed primarily to collect information regarding periodic fluctuations as they occur in nature, and secondly to provide hints of the factors involved. It is intended to test out any warrantable hypotheses that may be drawn from this data by critically controlled experiments. Two such subsidiary studies have already appeared, viz. "Some factors governing the emergence of gall midges" (Barnes, 1930), and "The sex ratio at the time of emergence and the occurrence of unisexual families in gall midges" (Barnes, 1931). A third, "A study of the segmentation of the antennae in gall midges," is now in the press.

Three avenues of approach to the problem have been taken into consideration. They are firstly, the *degree of infestation* of the crop in question. The two wheat blossom midges, *Contarinia tritici* (Kirby) and *Sitodiplosis mosellana* (Géhin), and a midge, *Dasyneura alopecuri* (Reuter), which lives on the seed of meadow foxtail grass, are being used in this phase. Secondly, there is the *degree of parasitism*, which is being studied in these three species and also the button top midge of basket willows, namely *Rhabdophaga heterobi* (H. Lw.). Thirdly, the *dates of emergence and number of broods* are being taken into account in the case of the above four midges and, in addition, the leaf-curling pear midge, *Dasyneura pyri* (Bouché), and the Arabis midge, *Dasyneura arabis* Barnes. In each case the observations are intended to be carried out over an initial period of 5 years.

II. INTRODUCTION.

The present paper deals with the infestation of a classical experimental field of wheat (Broadbalk) by the wheat blossom midges over a period of 5 years. The variety of wheat on this field is Standard Red. Biological information concerning the midges is dealt with as well as the fluctuations in degree of infestation of the wheat by the midges. The two species, *Contarinia tritici* (Kirby) and *Sitodiplosis mosellana* (Géhin) may be regarded as single brooded, although sometimes there is a partial second emergence of adults in the case of *C. tritici*.

A sample of wheat ears was gathered each year about 3 weeks after the crest of emergence of the adults. In this way the sample was picked at the time when the maximum number of larvae were present in the ears. It consisted of 500 ears, fifty being gathered from each of ten plots, all of which have been under different manurial treatment over a large number of years. The ten plots were chosen with a view to determining whether differential manuring affected the incidence of attack. The whole sample gave, on examination, the degree of infestation at the time when it was taken.

In order to determine the date for sampling, the larvae obtained from the previous year were kept and reared in an outdoor insectary at as nearly normal outside conditions as possible. The dates of emergence so obtained were checked up with field observations concerning the time of oviposition. This is allowable, since the midges are very short lived and oviposit as a rule the same day as they emerge.

The rearing of this material gave, in addition, a figure for the degree of parasitism at the time of emergence of host and parasites. This figure is called that of *relative or effective parasitism*, since any figures obtained by examining the midge larvae would only give the parasitism of the larvae and not the relative number of parasites to host which would be on the wing the following summer. This is due to the fact that nearly 10 months elapse between the time the midge larvae are fully fed and the emergence of the adults, and to the probability that weather conditions act differentially on the parasites and host. The fluctuations in relative parasitism will be dealt with in more detail when additional information has been amassed.

III. IDENTIFICATION.

The two species are very easily distinguishable after a little practice. The main morphological and colour differences have already been noticed (Barnes, 1928). The adults, larvae and eggs of *C. tritici* are golden yellow, while the same stages of *S. mosellana* are orange to carrot-red. The accompanying diagrammatic figures (Figs. 1 and 2) indicate the chief morphological characters of the two species.

IV. BIOLOGY AND DISTRIBUTION.

(*a*) *C. tritici*.

This species is on the wing from the time that normal flowering wheat ears first break their sheaths till about 5 weeks later, i.e. June to early July. Emergence takes place from pupae in the soil. The usual time of emergence is the evening. Hourly observations during the day have shown that 7 per cent. of the males and 10 per cent. of the females emerge between 9 a.m. (standard time) and 2 p.m., a further 30 per cent. of the males and 40 per cent. of the females appear between 2 p.m. and 8 p.m., and the remaining 63 per cent. of the males and 50 per cent. of the females emerge between 8 p.m. and 9 a.m.

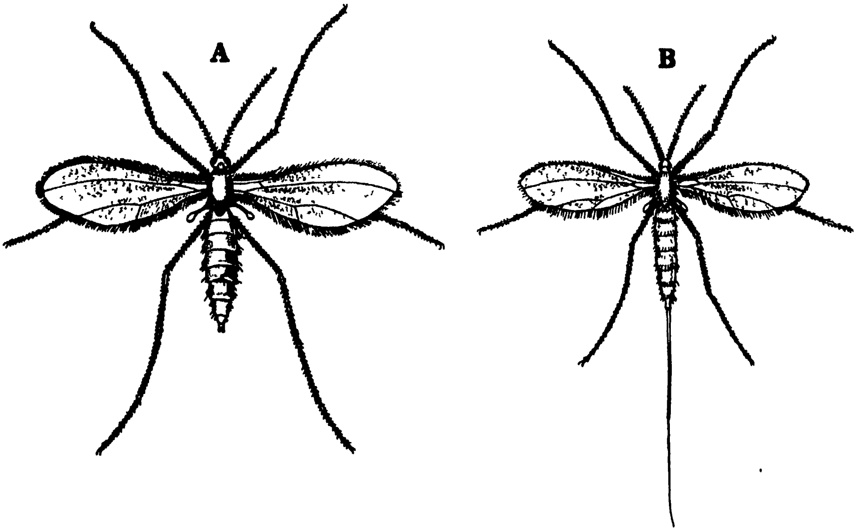

Fig. 1. Adult female midges: A, *Sitodiplosis mosellana* (Géhin); B, *Contarinia tritici* (Kirby). (After Wagner.) Reproduced by permission of the Imperial Institute of Entomology.

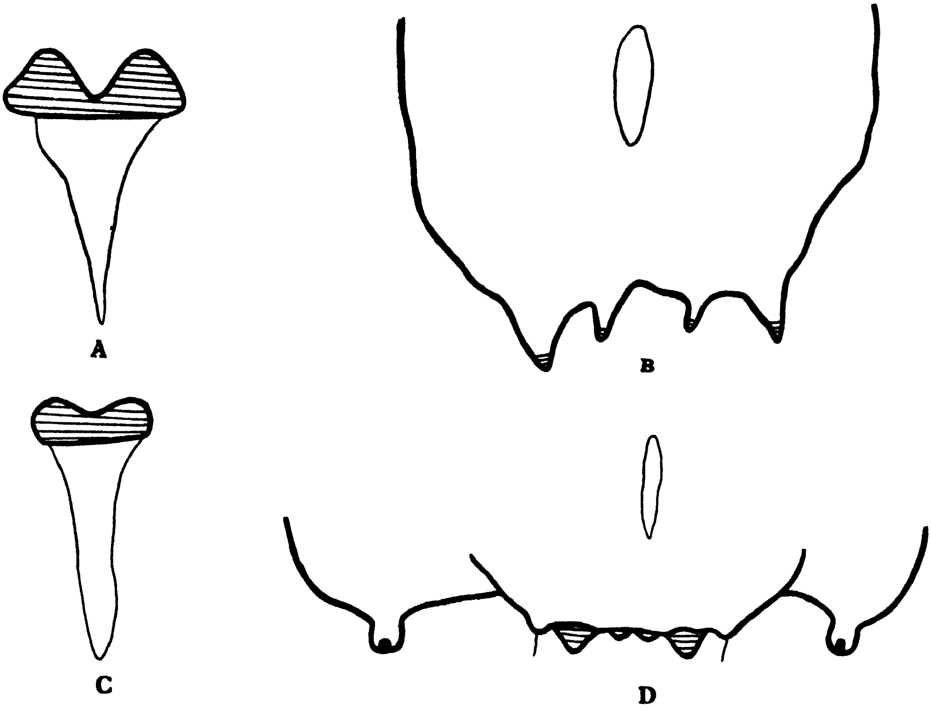

Fig. 2. Larvae of wheat blossom midges: A, anchor process of *Sitodiplosis mosellana*; B, anal extremity of *S. mosellana*; C, anchor process of *Contarinia tritici*; D, anal extremity of *C. tritici*. Reproduced by permission of the Imperial Institute of Entomology.

the following morning. The great bulk of these two last percentages emerges before 1 a.m.

As soon as emergence has taken place mating occurs and within a very few minutes the females are ovipositing on the ears. The males remain near the soil waiting for the females to emerge. The midges are short lived and their average life seems to be only a few hours if fertilisation and oviposition takes place, but up to 48 or even 72 hours if no males are available.

The eggs are pushed in between the glumes and are deposited on their inner faces. The ovipositor does not pierce any part of the floret. The larvae hatch in about 8 days or less if the weather conditions are favourable. They feed on the sap which is to form the kernel and so (except when only one or two larvae are present) prevent the formation of any corn; this is one of the biological characters which serve to separate this species from *S. mosellana*. The larvae also are to be found in considerable numbers in each attacked corn. This is the second distinguishing biological character. Table I shows the frequency of number of larvae per kernel. This number depends on the quantity of eggs laid in any one floret, this again being influenced by the amount of wind present at the time of oviposition. It also depends on when the sample is taken, for if the larvae have started leaving the kernels, the numbers will naturally be lowered.

Table I. *Frequency of* Contarinia tritici *larvae per corn of wheat,* 1927–31.

In 5560 occurrences

No. of larvae	Occurrences	No. of larvae	Occurrences	No. of larvae	Occurrences
1	212	24	55	47	7
2	246	25	62	48	5
3	243	26	54	49	2
4	246	27	34	50	2
5	277	28	30	51	1
6	270	29	40	52	4
7	281	30	30	53	1
8	272	31	25	54	1
9	305	32	24	55	1
10	305	33	26	56	2
11	322	34	12	57	3
12	267	35	13	58	2
13	269	36	14	64	1
14	280	37	12	65	1
15	215	38	8	66	1
16	228	39	11	68	1
17	166	40	4	69	1
18	165	41	9	75	1
19	129	42	6	81	1
20	98	43	4	83	1
21	96	44	5	85	2
22	74	45	5	88	1
23	64	46	4	91	1

The larvae have attained their maximum size by about 4 weeks after oviposition and then, unless the weather is very dry, they migrate by "jumping" to the soil. Scarcely any larvae remain in the ears, as is shown from the examination of ears plucked at the time of harvest. They remain in the surface

3 in. of soil in a semi-quiescent state until the following spring. Then they become more active and pupate about 8 days to a fortnight previous to the ears of wheat breaking their sheaths. Emergence takes place as previously stated.

This is the normal life cycle. Occasionally, however, a partial emergence takes place in August and September the same year as the eggs are laid. Something under 10 per cent. of the total number of adults emerge in this way. This emergence in September has also been noticed by Kirby (1798). Whether these individuals can exist on wild grasses or not is being investigated. Wagner (1866) states that under such circumstances they have no choice but to oviposit on couch grass (*T. repens*). He also states that midges emerging before the wheat flowers appear oviposit on the more precocious rye. This species is also reputed to attack barley.

While ovipositing the midges are very engrossed in their work and are exceptionally vulnerable. The Empid fly, *Tachydromia pallidiventris* Mg. (kindly identified by Mr J. E. Collin), was caught while eating egg-laying females on Broadbalk, Harpenden, in June and July, 1927. Adult *Trombidium* sp. have also been observed sucking the abdomens of this midge while on the ears of wheat. Curtis (1860) quotes *Empis livida* Linn. as being an enemy of this species.

(*b*) *S. mosellana.*

The life cycle of this species is almost identical with that of *C. tritici*. The exact method by which the larvae leave the ears is not yet known. There are, however, three points of difference which may be mentioned. Firstly, the larvae feed on the surface of the kernel as it is being formed, but do not enter it, and so cause shrunken corn. Secondly, it is usual to find only one or two larvae present on one kernel. This is clearly shown in Table II.

Table II. *Frequency of* Sitodiplosis mosellana *larvae per corn of wheat,* 1927–31.

In 10,605 occurrences

1	larva was	found	on a single corn	7655	times
2	larvae were	,,	,,	2011	,,
3	,,	,,	,,	634	,,
4	,,	,,	,,	199	,,
5	,,	,,	,,	59	,,
6	,,	,,	,,	29	,,
7	,,	,,	,,	13	,,
8	,,	,,	,,	3	,,
9	,,	,,	,,	1	,,
10	,,	,,	,,	1	,,

The third difference in life history is that no secondary partial emergence has yet been observed. This species has been observed to oviposit on slender foxtail grass (*Alopecurus myosuroides*). Other workers have stated that this species also attacks rye, barley and oats. Experiments are being undertaken to determine the range in host plants of both *C. tritici* and *S. mosellana*. Adult *Trombidium* sp. have been observed eating ovipositing females at Harpenden.

Occasionally both species of larvae are found in a floret. The frequency of this occurrence seems to depend on the intensity of the population as is shown in Table III.

Table III. *Frequency of larvae of* Contarinia tritici *and* Sitodiplosis mosellana *occurring in the same floret of wheat.*

Year	Total occurrences of *C. tritici* and *S. mosellana*	No. of times when both were found in the same floret	Percentage corn attack by both species
1927	811	3	3·2
1928	1689	6	6·5
1929	1868	10	7·7
1930 F*	2243	26	17·1
1930	3821	38	18·0
1931	5733	37	21·4

* 1930 F = sample taken from the first crop after fallow for 2 years. 1930 = sample taken from third successive crop after fallow for 2 years. See section VI (iii).

(c) *Distribution.*

Until very recently writers on wheat midges did not differentiate between the two species although Wagner (1866) made the differences quite clear. It was advisable to attempt to find out the distribution of the two species in the British Isles. Previously confirmed records are as follows: both species in Cambridge (1927), Kent (1926); *S. mosellana* only in Pembrokeshire (Anon. 1908), Norfolk (1927), Suffolk (1927), Rutland (A.R., 1927), Sussex (Brit. Mus. Nat. Hist. specimens), Leicester (A.R., 1927), Lincoln (A.R., 1927), Nottingham (A.R., 1927) and Derby (A.R., 1927). Accordingly a systematic survey has been started. Samples from the following counties in England have revealed larvae of both species in 1930: Bedford (H.C.F.N.), Devonshire (W.E.H.), Durham (R.A.H.G.), Gloucester and Hereford (C.L.W.), Monmouth (H.W.T.), Northamptonshire (N.C.), Somerset (C.L.W.) and Yorkshire (T.H.T.). But one sample from Northumberland (R.A.H.G.) gave only a 2 per cent. ear attack by *S. mosellana*, while another gave completely negative results for both species. Likewise both species were absent from a sample from Cumberland (R.A.H.G.). Three samples from Glamorgan (H.W.T.) showed both species present in each, but one sample from Cardigan (J.R.W.J.) and another from Denbigh (W.M.D.) only revealed *S. mosellana*. From Scotland one sample has been received, from Kincardine (G.M.), and *S. mosellana* only was present. In eight samples from Co. Armagh, Co. Derry, Co. Down, Co. Fermagh and Co. Tyrone (per S.P.M.), *S. mosellana* was found in one sample from each of the following three counties, Armagh, Derry, and Fermagh. *C. tritici* was found in none of the Irish samples. It will be of some importance to continue this survey in order that one can find out definitely if *C. tritici* is really absent from Ireland, Scotland, and parts of Wales and the north of England, and if *S. mosellana* is only sparsely scattered in Ireland.

V. Oviposition.

At Wye, Kent, observations were made in 1926 to determine at what time in the day oviposition takes place. Midges of both species were found to be most numerous on the wheat ears about 7.30 p.m. (standard time). All times are given as standard time. Oviposition, however, started late in the afternoon and continued until about 2 a.m., at which time the dew came. After this and during the day midges were to be found on the stalks and leaves of the wheat. It was noticed that there is a definite sleeping posture.

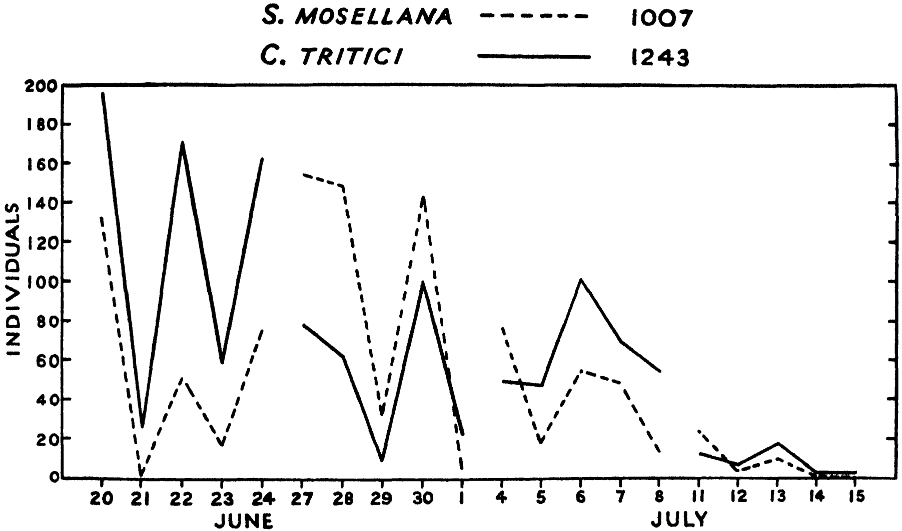

Fig. 3. The numbers of *C. tritici* and *S. mosellana* ovipositing on Broadbalk, 1927.

Observations were made at Rothamsted in 1927 to determine the duration of the oviposition period. The method adopted was to walk round the different plots of wheat on Broadbalk continuously from 6.45 to 8.15 p.m. and to collect, with a wet paint brush and tubes containing alcohol, all the females observed ovipositing. These were afterwards counted; it is to be understood that the numbers so obtained are only relative. This procedure was carried out 5 days a week for 4 weeks. In this way it was possible to calculate the duration of the egg-laying period. The time chosen was that when the greatest number of midges would be ovipositing. This experiment was started the day large numbers were first seen, i.e. about the crest of oviposition or emergence.

Fig. 3 shows the results of these observations. It will be seen that the crest of oviposition of *S. mosellana* is a week later than that of *C. tritici*. This is corroborative of the fact that the larvae of *C. tritici* prevent corn formation, while those of *S. mosellana* feed on the developing corn.

The fluctuations in numbers from day to day are very marked. Table IV shows the effect of weather on the numbers ovipositing. It can also be shown that there is a partial correlation with the direction and strength of the wind and the temperature of the soil and air. Humidity and barometric pressure should also be taken into account, but, from the present observations, do not seem to be of major importance. Sultry nights seem to be the most favourable to the ovipositing midges.

Table IV. *Effects of weather on numbers of females ovipositing.*

		Nos. of ovipositing females of	
Date	Weather, 6.45 p.m. to 8.15 p.m. (standard time)	*Contarinia tritici*	*Sitodiplosis mosellana*
June 20	Cloudy, sultry, scarcely any breeze	196	132
,, 21	Cloudy, cold, south-west gusty wind	26	1
,, 22	Sunny, not so cold as last night, slight breeze	170	51
,, 23	Bright sunny intervals, heavy clouds, cool, strong to light south-west wind	59	17
,, 24	Dull, sultry, followed by heavy thunder shower	162	75
,, 27	Dull, sultry, rain threatening, very heavy shower 5.45–6.10 p.m.	78	154
,, 28	Sunny and cloudy, no breeze, thundery but no rain	62	148
,, 29	Heavy rain, very wet, cool breeze	9	32
,, 30	Bright sun, no breeze, heavy rain till 1 p.m., none after	100	143
July 1	Sun at intervals, cool, strong breeze	22	5
,, 4	Dull, sultry, hot	49	77
,, 5	Sunny, sultry, slight breeze	47	18
,, 6	Dull, sultry, hot, with slight drizzle at intervals	101	54
,, 7	Bright sun, cool, no breeze, heavy thunder showers in afternoon	69	48
,, 8	Dull, sultry, thunder shower 5.30 p.m., ears still wet, slight breeze at 7.45 p.m.	54	13
,, 11	Dull, sultry, hot, heavy thunder shower 4–6 p.m.	12	24
,, 12	Scotch mist, slight drizzle, hot, heavy clouds, rain late afternoon	6	3
,, 13	Dull, heavily overcast, fine, rather cooler	17	9
,, 14	Dull, overcast, wet, stopped raining about 7.30 p.m.	2	1
,, 15	Dull, slight breeze, cool	2	0

At the height of oviposition literally swarms of the yellow (*C. tritici*) and orange (*S. mosellana*) midges can be seen hovering in clouds over wheat fields. The actual date sometimes varies from one year to another: for instance Shirreff (1873) noticed midges on the wing on June 21st in 1829 (*sic*), from June 10th ("wheat flies first seen tonight") to June 17th ("flies innumerable") in 1871, and from June 29th (first seen), July 4th ("great numbers") to July 23rd (last seen) in 1872. The observations of the writer tend to show that the actual date does not vary very much as a rule. Thus in 1926 the crest of oviposition was about June 30th to July 1st at Wye, Kent; in 1927 it was on June 20th on Broadbalk, Hertfordshire. The crest of emergence in 1929 in this latter locality was on June 20th, in 1930 June 15th and in 1931 June 16th.

VI. SAMPLING ON BROADBALK.

(i) *Methods.*

Since oviposition continues for something over 5 weeks and the larvae remain in the ears only about 4 weeks, it is obvious that samples taken on any one day cannot give the total infestation of the crop. It was decided, owing to the vast labour of examining the wheat ears, that sampling could only be done once a season and to take the sample when the maximum number of larvae would be present at any one time. This was determined as about 3 weeks after the crest of oviposition. Thus in 1927 the sample of wheat ears was taken on July 13th, 23 days after the crest of oviposition on June 20th. The crest of *C. tritici* was chosen rather than that of *S. mosellana* as the larvae of the former spend a slightly shorter time in the ears.

The plan of gauging the correct day for sampling by the method of finding the crest of oviposition proved to be too exacting and so another method was evolved. From 1928 onwards the larvae obtained in the sampling were reared in the insectary. The daily emergences observed were checked with field observations and so the crest of emergence was found. As the midges oviposit almost immediately after emerging, it was decided to consider the date of the crest of emergence as coinciding with that of the crest of oviposition. In this way the correct date for sampling was determined. Table V shows the method of obtaining the correct date for sampling.

Table V. *Method of obtaining the correct date for sampling.*

Year	Crest of oviposition or emergence	Allow about 3 weeks interval	Date for sampling
1927	June 20	23 days	July 13
1928	—	—	„ 16
1929	„ 20	25 „	„ 15
1930	„ 15	23 „	„ 8
1931	„ 16	23 „	„ 9

The annual sample was taken on Broadbalk and consisted of 500 ears of wheat. This field has been a classical experiment on differential manuring for many years, and advantage was taken of this fact. The whole sample was divided into ten smaller samples of fifty ears each, and it was decided to choose ten of the wheat plots with a view to investigating whether differential manuring of the plant affected the incidence of attack. The following plots were selected.

Plot	Treatment
2	Dung
3	Unmanured (since 1839)
5	Complete minerals
8	Complete minerals + 618 lb. sulphate of ammonia (S./A.)
10	412 lb. S./A. only
11	412 lb. S./A. + 3½ cwt. super
12	" " + 366 lb. S./Soda
13	" " + 200 lb. S./Potash
14	" " + 280 lb. S./Magnesia
16	Complete minerals + 550 lb. nitrate soda

These plots have been manured each year in the same way since 1847, excepting plot 16 which has only received this dressing since 1906.

The actual process of sampling was to walk up one side of each plot, across the top, and down the other side, picking one ear of wheat at given intervals. The frequency of picking the ears was calculated roughly to enable fifty ears to be plucked by the time each plot had been walked round once. It is admitted that the sample was only representative of the outside arm's length of each plot. The ears from each plot were put into separate paper bags and examined in detail as soon as possible. This examination has taken on an average 4 hours a day for a fortnight of 6 working days or about 50 hours to complete. The procedure has been for the writer to examine ear by ear and floret by floret dictating to an assistant who writes down the numbers. It is impossible to work continuously for more than about 2 hours, as the eye strain of the examiner and the monotony for the assistant is too great. It takes just over 5 min. to examine one ear.

(ii) *Effect of differential manuring on incidence of attack.*

Fig. 4 shows the percentage kernel infestation by the two species of midges on ten differently manured plots over a period of 5 years. This damage is that suffered by the crop at the time of sampling. Acting on Dr R. A. Fisher's advice it is proposed at some future date to try to obtain data as to the weight of corn from wheat ears that have been attacked compared with that from unattacked ears. With such data and by the use of regression curves it should be possible to find out whether there is any compensating effect or not by the time of harvest. There are two possibilities: the presence of midge larvae on certain kernels in an ear may result in a temporary draining of the sap going to form the other kernels and so a general decrease in weight of corn; on the other hand, their presence and loss in weight of attacked kernels might be compensated for by an increase in the weight of the unattacked ones.

The actual figures of percentage ear and kernel attack, number of larvae present, number of kernels attacked, as well as the total number of kernels, spikelets and blind spikelets at the two extremities of the ear can be found in Tables VIII–XIII.

No constant significant difference in attack due to manuring has been found, either in the amount of nitrogen given to the plant or the amount of

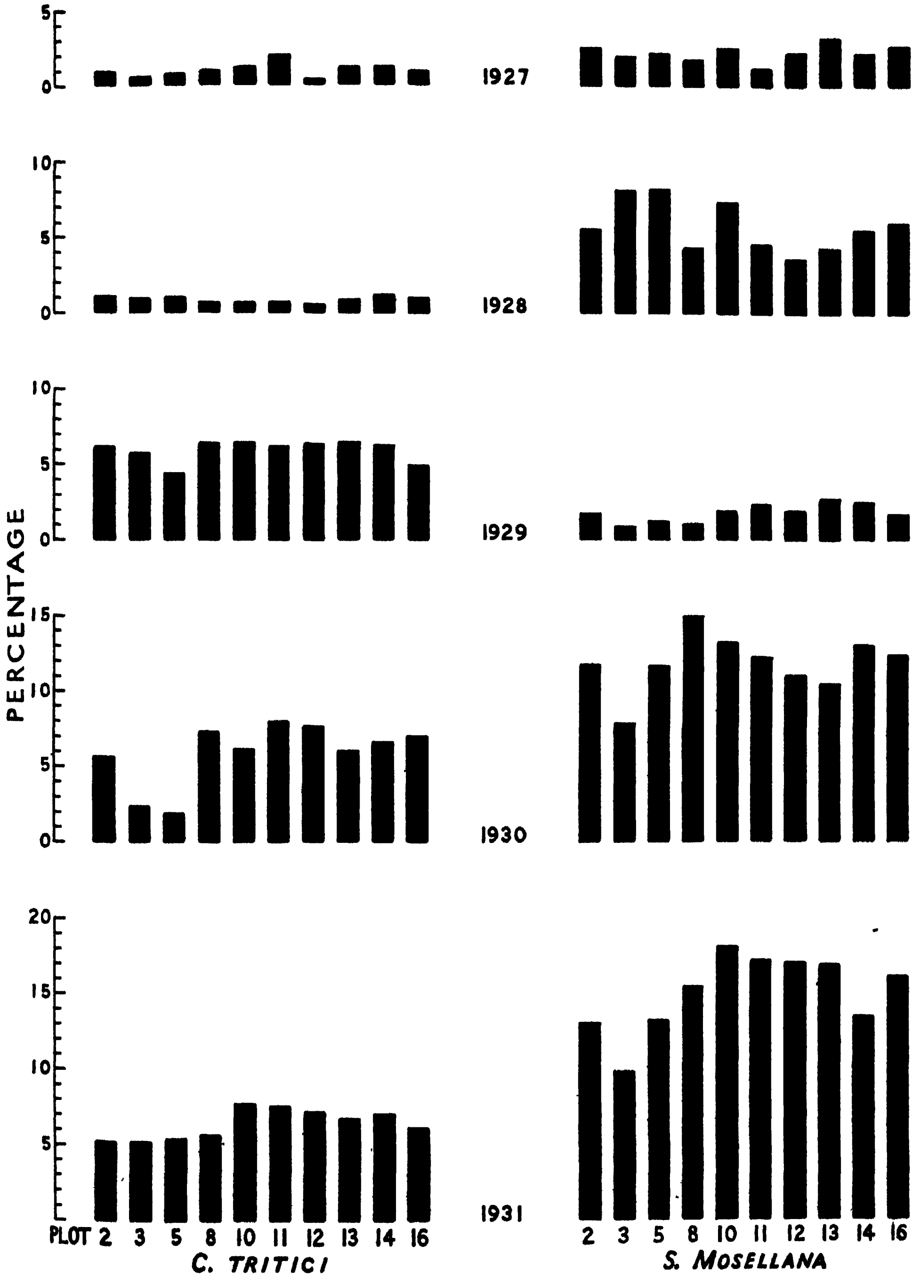

Fig. 4. Percentage kernel infestation by *C. tritici* and *S. mosellana* on ten plots of Broadbalk, 1927–31. The histograms for 1930 represent the average of the two samples taken in that year.

the mineral constituents of the treatment. Although the manurial treatments do affect the time of flowering to some extent there is no corresponding difference in attack by either of the midges. This is to be expected, as the time of flowering is not appreciably altered in relation to the duration of the time of oviposition.

(iii) *General inferences.*

Since the manurial treatment does not appreciably affect the intensity of attack, one can consider the ten samples from the different plots as composing one replicated sample for the whole field. Table VI gives the degree of infestation for the whole of Broadbalk. It can be seen that in the case of *C. tritici* after 2 years, when the attack was low (about 1 per cent.), there was a decided increase in intensity which remained nearly constant (about 6 per cent.). The fluctuations of *S. mosellana* were of a different nature. In the 5 years under consideration the attack varied from about 2 per cent. in 1927 to about 5½ per cent. in 1928, then decreased to about 2 per cent. in 1929, and rose to about 11½ per cent. in 1930 to 15 per cent. in 1931. It is evident that each of these species responds in a different manner to the factors at work.

Table VI. *Degree of infestation or percentage kernel attack by* Contarinia tritici, *and* Sitodiplosis mosellana *on Broadbalk*, 1927–31.

	1927	1928	1929	1930	1931
		C. tritici			
No. of larvae	1,780	2,195	19,265	15,255 21,934 F	19,273
No. of attacked kernels	239	203	1,434	1,157 1,630 F	1,701
Percentage attacked	0·95	0·79	5·9	5·4 6·3 F	6·4
		S. mosellana			
No. of larvae	715	2,043	587	3,659 3,832 F	6,027
No. of attacked kernels	541	1,486	434	2,664 2,856 F	4,032
Percentage attack	2·2	5·7	1·8	12·6 10·8 F	15·0
No. of kernels in sample (500 ears)	24,555	25,735	24,342	21,115 25,940 F	26,857

It may be pointed out that in 1926 and 1927 one-half of Broadbalk was left fallow and in 1928 and 1929 the other half was left fallow. Consequently the sample of 1928 was of the first crop of wheat after 2 years fallow, that of 1929 of the second crop after 2 years fallow. Two samples were taken in 1930: one from the top half of the field, being of the third crop after 2 years fallow, and the other (F) from the bottom half of the field[1], being of the first crop after

---

[1] In this case twenty-five ears constituted a sample from a plot instead of fifty as in each of the other samples. In Table XII, the actual figures are used, but in Table VI they are doubled for sake of uniformity.

2 years fallow. In 1931 the sample was taken from the whole field irrespective of the fallowing operations. It will be seen that the numbers of kernels in the two samples taken of the first crop after 2 years fallow were 25,735 and 25,940, the number in that of the second crop 24,342, and in that of the third crop 21,115. This indicates a steady decrease in the yield following successive cropping.

Another inference which may be drawn is that such a study as this only begins to show interesting results after a period of 5 years, and that it is highly desirable that it should be continued for a much longer period.

It would also be of great practical importance if this study, instead of being confined to one variety of wheat (Standard Red) in one locality, could be made to include several varieties from various parts of the British Isles. The sampling method now adopted is one that could be put on a routine basis and not suffer very much. But few individuals would have time enough to carry out this method. Bearing this in mind the data obtained was examined in order to see whether by taking the percentage ear attack one could estimate the percentage kernel attack. It was found that in the case of *C. tritici* up to about 65 per cent. of ear attack represented up to 3 per cent. kernel attack, and from about 70 to 92 per cent. ear attack represented 4–9 per cent. kernel attack. In the case of *S. mosellana* up to 65 per cent. ear attack represented up to about 3 per cent. kernel attack, about 70–90 per cent. ear attack represented 4–7 per cent. kernel attack, and from 90–100 per cent. ear attack represented 8 per cent. kernel attack upwards. It will be seen that by finding percentage attack of wheat ears one could only estimate percentage kernel attacks very roughly, perhaps three divisions, viz. below 3 per cent., between 4 and 7 per cent. and above 8 per cent.

VII. PARASITES.

(i) *Identification.*

Various Hymenopterous parasites are known to attack *C. tritici* and *S. mosellana* (for previous records see Barnes (1927)). Those reared in 1929 from the larvae collected in the samples in 1928 were submitted to the late Dr J. Waterston with the following results. Quoting from a letter, dated November 9th, 1929: "From *C. tritici* you have reared *Isostasius punctiger* Nees, and a species of *Synopeas* which may be *sosis* of Walker. From *S. mosellana* you have bred *Macroglenes penetrans* Kirby...and a very remarkable inostemmine species, which is probably referable to the genus *Brachinostemma* but if so it does not appear to correspond with any of the species at present described in that genus. With this peculiar parasite you have also two apparently distinct species of *Platygaster*, which at present I cannot determine." By the untimely death of Dr Waterston this side of the study was put in abeyance for the time being.

The biology of these parasites remains to be investigated and the exact rôle they play to be determined.

(ii) *Methods and results.*

The midge larvae taken from the samples from Broadbalk have been reared in an outdoor insectary in lamp glasses over pots containing fibre and soil. Besides obtaining the correct date for sampling, this breeding has given figures for the emergence of the parasites. Up to the present all the parasites emerging from each midge species have simply been noted as parasites, no attempt being made to separate the different species. All the material has been kept, however, with a view to obtaining the total number in any one year of each species of parasite. The accompanying graph (Fig. 5) shows the daily emergence in 1930 of the parasites compared with that of the host midge, in this case *C. tritici*. It will be seen that the emergence of the parasites is slightly after that of the host. This appears to be the normal occurrence.

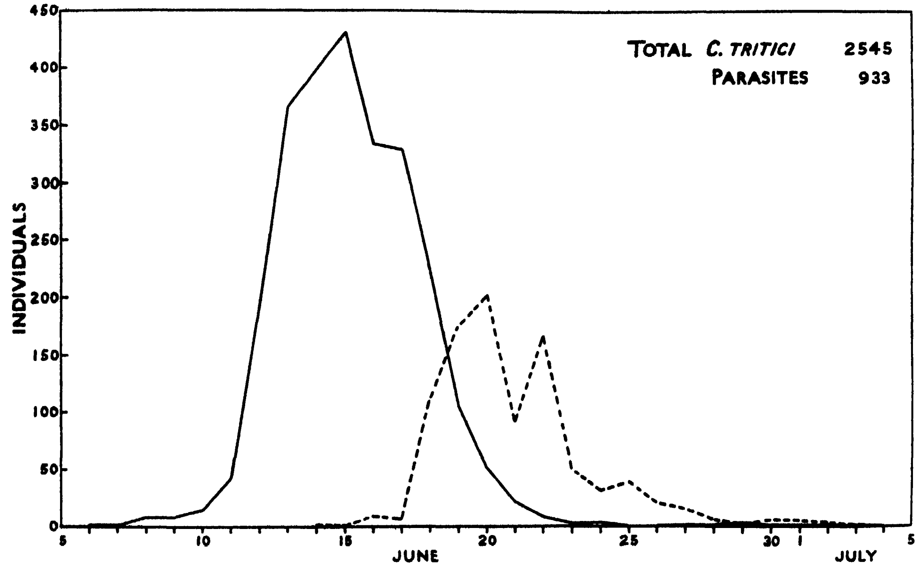

Fig. 5. Daily emergence in 1930 of *C. tritici* and its parasites in the insectary.

The advantage gained by this rearing method is that a figure for the *relative or effective parasitism* is obtained. That is to say the number of parasites which have survived to the imaginal state compared with the number of midges which will be causing the season's attack. It does not give any idea of the number of larvae killed by the parasites.

Acting on the advice of Mr J. C. F. Fryer, it is proposed to examine the relative numbers of midges and parasites emerging from the soil actually on Broadbalk to see whether the figures obtained in the insectary bear any relation to those obtaining in the field.

Table VII shows the relative parasitism figures in the insectary for the years 1929–31, obtained from the previous years samples. It should be explained that all the material of *S. mosellana* collected in 1928 from the ten plots on Broadbalk was treated as one sample and this resulted in there being one figure for parasitism in 1929. Likewise all the material collected in 1929 was divided into two samples giving two parasitism figures in 1930. In 1930 the material was divided into four samples.

The same applies in the case of *C. tritici* except that in 1929 the material was divided into five samples giving five figures of parasitism in 1930.

Table VII. *Relative parasitism figures, obtained by breeding in the insectary, of* Contarinia tritici *and* Sitodiplosis mosellana *in* 1929–31.

	Samples of relative or effective % parasitism					
Year	1	2	3	4	5	Average
			C. tritici			
1929	10	—	—	—	—	10
1930	5	27	28	39	31	27
1931	38	69	57	54	—	53
			S. mosellana			
1929	73	—	—	—	—	73
1930	40	45	—	—	—	43
1931	69	78	98	92	—	85

It will be seen that there is a significant difference in degree of relative parasitism from year to year.

The histograms (Fig. 6) show the percentage kernel attack on Broadbalk wheat by the two midges for the 5 years 1927–31 and the relative parasitism percentage for 3 years.

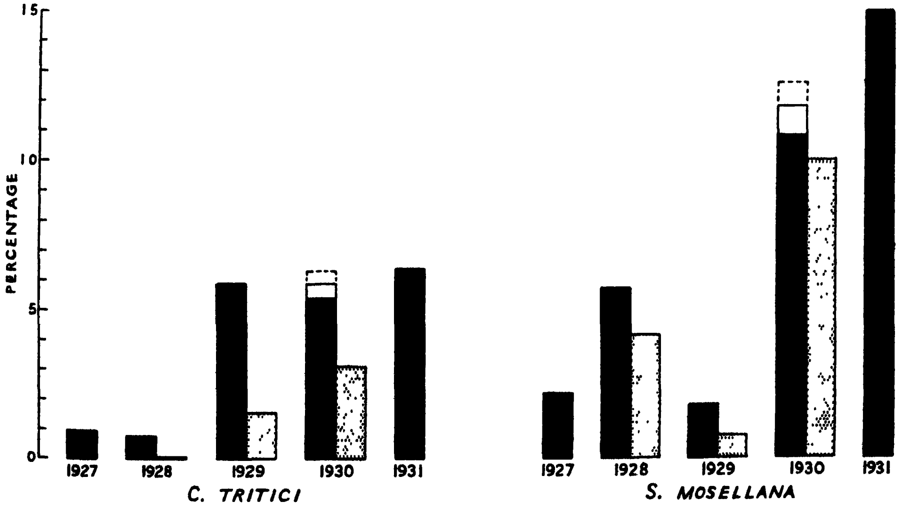

Fig. 6. The percentage kernel attack (solid) on Broadbalk wheat by *C. tritici* and *S. mosellana*, 1927–31 and the relative parasitism percentage for 1928–30 (mechanically stippled).

It is proposed to leave the discussion of the parasitism of these two midges until further data has been acquired.

VIII. SUMMARY.

1. A project is explained in which the fluctuations of insect populations in the field are to be studied on a quantitative basis. Three avenues of approach are to be used; the degree of infestation or intensity of attack by the larvae, the degree of parasitism and the dates of emergence (and number of broods). The gall midges are to be used for this study.

2. In this first contribution the wheat blossom midges, *C. tritici* and *S. mosellana*, on Broadbalk have received attention. The fluctuations in intensity of attack over a period of 5 years have been recorded. In the same way the degrees of parasitism for 3 years are noted. The bionomics have been studied over a period of 6 years.

3. The methods employed in sampling and breeding have been explained in detail.

4. A full discussion of the results obtained is being held over until the whole project, entailing similar studies on four additional species of gall midges, has been completed. This should be in 2 years time.

IX. ACKNOWLEDGMENTS.

I should like to thank Dr A. D. Imms, Dr R. A. Fisher and Mr J. C. F. Fryer for their help in discussing various aspects of the work. In addition, I am indebted to all the gentlemen who have so kindly sent me samples of wheat. I wish especially to thank all those who have helped me in the somewhat tedious task of taking and examining the samples and also by checking up all the data. My thanks are also due to Mr A. D. Dunkley for preparing Figs. 3–6 and to the Imperial Institute of Entomology for permission to reproduce Figs. 1 and 2 from the *Bulletin of Entomological Research.*

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Table VIII. *Broadbalk wheat*, 1927. *Midge infestation in ears on July* 13*th.*

Plot	% ear attack		% ear attack by both midges	Total spikelets in 50 ears	No. of kernels in 50 ears	No. of kernels infested		% kernel attack		Total larvae in 50 ears		Av. no. larvae per kernel		Total blind spikelets in 50 ears
	T.	*M*				*T.*	*M*	*T.*	*M.*	*T.*	*M.*	*T.*	*M.*	
2	36	54	74	1077	2662	23	63	0·9	2·4	158	74	6·9	1·2	107
3	16	32	46	910	1722	9	32	0·5	1·9	107	33	11·9	1·0	155
5	20	38	52	963	1869	13	38	0·7	2·1	115	53	8·7	1·4	151
8	40	50	66	1017	2533	23	42	0·9	1·7	106	51	4·3	1·2	113
10	52	64	86	1032	2699	33	65	1·2	2·5	193	86	5·8	1·3	84
11	60	40	72	1029	2582	48	30	1·9	1·2	276	43	6·0	1·0	97
12	12	54	58	1028	2459	8	55	0·3	2·2	54	65	6·9	1·2	109
13	38	56	85	1077	2574	28	82	1·1	3·2	226	110	8·0	1·3	131
14	44	52	87	1035	2752	32	61	1·2	2·1	335	93	10·5	1·5	100
16	30	60	81	1081	2703	22	73	0·8	2·7	210	107	9·5	1·5	127

Table IX. *Broadbalk wheat*, 1928. *Midge infestation in ears on July* 16*th.* (*First wheat crop after* 2 *years fallow.*)

Plot	% ear attack		% ear attack by both midges	Total spikelets in 50 ears	No. of kernels in 50 ears	No. of kernels infested		% kernel attack		Total larvae in 50 ears		Av. no. larvae per kernel		Total blind spikelets in 50 ears
	T.	*M.*				*T.*	*M.*	*T.*	*M.*	*T.*	*M.*	*T.*	*M.*	
2	26	74	76	1136	2478	25	136	1·0	5·5	224	198	8·9	1·5	119
3	30	96	96	1092	2612	21	211	0·8	8·1	248	288	11·8	1·3	107
5	28	90	92	1123	2669	27	219	1·0	8·2	367	317	13·6	1·5	116
8	20	80	80	1146	2557	15	115	0·6	4·3	130	156	8·7	1·4	112
10	22	90	92	1108	2716	15	198	0·6	7·3	131	274	8·7	1·4	95
11	22	66	68	1121	2573	15	116	0·6	4·6	150	150	10·0	1·3	111
12	18	74	78	1140	2565	14	92	0·5	3·6	133	107	9·5	1·2	109
13	28	72	76	1136	2548	20	110	0·8	4·3	249	155	12·1	1·4	126
14	32	78	82	1108	2441	28	135	1·1	5·5	289	185	10·0	1·4	119
16	28	84	88	1128	2576	23	154	0·9	6·0	274	213	11·9	1·4	117

Table X. *Broadbalk wheat*, 1929. *Midge infestation in ears on July* 15*th*. (*Second successive wheat crop after* 2 *years fallow.*)

Plot	% ear attack		% ear attack by both midges	Total spikelets in 50 ears	No. of kernels in 50 ears	No. of kernels infested		% kernel attack		Total larvae in 50 ears		Av. no. larvae per kernel		Total blind spikelets in 50 ears
	T.	*M.*				*T.*	*M*	*T.*	*M*	*T.*	*M.*	*T.*	*M.*	
2	88	48	88	1056	2617	161	44	6·2	1·7	2379	69	14·1	1·6	92
3	78	28	84	1042	2611	149	22	5·7	0·8	2675	31	17·8	1·4	100
5	70	40	78	1031	2190	96	29	4·4	1·2	1338	36	13·9	1·2	135
8	78	18	78	1068	2142	137	22	6·4	1·0	1633	30	11·9	1·4	143
10	90	54	96	1053	2460	158	47	6·4	1·9	2175	69	13·8	1·5	111
11	90	56	94	1036	2385	145	55	6·1	2·3	1987	72	13·7	1·3	113
12	86	50	94	1065	2387	151	45	6·3	1·9	1937	56	12·9	1·2	119
13	88	70	96	1103	2611	166	70	6·4	2·7	1918	88	11·6	1·3	109
14	82	48	92	1074	2259	141	56	6·2	2·5	1683	77	11·9	1·4	141
16	74	44	84	1100	2680	130	44	4·9	1·6	1540	59	11·8	1·3	102

Table XI. *Broadbalk wheat*, 1930. *Midge infestation in ears on July* 8*th*.

(*Third successive wheat crop after* 2 *years fallow: top half.*)

Plot	% ear attack		% ear attack by both midges	Total spikelets in 50 ears	No. of kernels in 50 ears	No. of kernels infested		% kernel attack		Total larvae in 50 ears		Av. no. larvae per kernel		Total blind spikelets in 50 ears
	T.	*M.*				*T.*	*M.*	*T.*	*M.*	*T.*	*M.*	*T.*	*M.*	
2	78	96	100	1105	2244	120	249	5·3	11·1	1529	354	12·7	1·4	151
3	28	86	86	1022	1928	29	135	1·5	7·0	338	171	11·7	1·3	163
5	22	90	94	1029	1758	20	215	1·1	12·2	260	303	13·0	1·4	179
8	92	98	100	1167	2158	164	369	7·6	17·1	2117	544	13·0	1·5	198
10	86	100	100	1151	2164	131	287	6·1	13·3	1821	382	13·9	1·3	174
11	80	100	100	1106	2130	142	291	6·7	13·7	2027	385	14·3	1·3	171
12	88	98	100	1089	2077	150	255	7·2	12·3	2073	354	13·8	1·4	170
13	80	98	100	1160	2265	138	252	6·1	11·1	1707	355	12·4	1·4	170
14	76	100	100	1127	2149	126	287	5·9	13·4	1738	379	13·8	1·3	179
16	74	100	100	1167	2242	137	324	6·1	14·5	1645	432	12·0	1·3	194

Table XII. *Broadbalk wheat*, 1930. *Midge infestation in ears on July* 8*th*.

(*First wheat crop after* 2 *years fallow: bottom half.*)

Plot	% ear attack		% ear attack by both midges	Total spikelets in 25 ears	No. of kernels in 25 ears	No. of kernels infested		% kernel attack		Total larvae in 25 ears		Av no. larvae per kernel		Total blind spikelets in 25 ears
	T.	*M.*				*T.*	*M.*	*T.*	*M.*	*T.*	*M.*	*T.*	*M.*	
2	80	96	100	583	1482	89	181	6·0	12·2	1318	263	14·8	1·5	59
3	76	100	100	564	1322	41	112	3·1	8·5	582	152	14·2	1·4	61
5	64	100	100	575	1233	33	136	2·7	11·0	460	172	13·9	1·3	77
8	88	100	100	604	1302	90	164	6·9	12·6	1290	234	14·3	1·4	78
10	72	96	100	587	1385	83	180	6·0	13·0	1341	239	16·2	1·3	67
11	88	92	100	576	1373	123	145	9·0	10·6	1659	200	13·5	1·4	64
12	88	92	100	575	1201	98	115	8·2	9·6	1040	155	10·6	1·3	87
13	72	92	98	576	1134	66	109	5·8	9·6	668	143	10·1	1·3	103
14	76	100	100	584	1235	89	154	7·2	12·5	1190	187	13·4	1·2	88
16	84	92	96	590	1303	103	132	7·9	10 1	1419	171	13·8	1·3	74

Table XIII. *Broadbalk wheat*, 1931. *Midge infestation in ears on July* 9*th*.

Plot	% ear attack		% ear attack by both midges	Total spikelets in 50 ears	No. of kernels in 50 ears	No. of kernels infested		% kernel attack		Total larvae in 50 ears		Av. no. larvae per kernel		Total blind spikelets in 50 ears
	T.	*M.*				*T.*	*M.*	*T.*	*M.*	*T.*	*M.*	*T.*	*M.*	
2	84	94	96	1161	3189	166	414	5·2	13·0	2115	625	12·7	1·5	74
3	76	94	96	1079	2305	119	226	5·2	9 8	1441	329	12·1	1·5	140
5	74	100	100	1139	2841	151	376	5·3	13 2	1844	589	12·2	1·6	80
8	90	100	100	1179	2510	140	384	5·6	15·3	1830	596	13·1	1·6	149
10	90	96	100	1147	2566	197	461	7·7	18·0	2336	741	11·9	1·6	135
11	94	98	100	1136	2911	218	497	7·5	17·1	2329	692	10·7	1·4	89
12	90	100	100	1141	2911	210	496	7·2	17·0	2139	760	10·2	1·5	90
13	90	100	100	1162	2465	164	413	6·7	16·8	1788	593	10·9	1·4	149
14	90	98	100	1142	2440	170	326	7·0	13·4	1867	469	11·0	1·4	146
16	86	100	100	1137	2719	166	439	6·1	16·1	1584	633	9·5	1·4	125

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ON THE GALL MIDGES INJURIOUS TO THE CULTIVATION OF WILLOWS

I. THE BAT WILLOW GALL MIDGE (*RHABDOPHAGA TERMINALIS* H.LW.)

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(With Plates XII and XIII.)

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1. Introduction.

The larvae of several species of gall midges (Cecidomyidae) are responsible for serious damage done to commercially grown willows. They may be conveniently divided into three groups according to the type of injury they cause. Firstly, there are those which destroy the terminal bud or growing point, resulting in the production of side shoots. Secondly, there are those living in the stems or rods. As a consequence rot is liable to develop and this entails in some cases a reduction in the number of sets which normally would be cut from healthy rods, in other cases weak points in the rods when being worked up into baskets, and in others unhealthy stubs. Thirdly, there are those which cause galls to form on the leaves. The presence of this type of damage does more to reduce the value of the standing crop in the eyes of possible buyers than actual injury to the rods, except very occasionally in years of great abundance when the plants suffer from the diminished surface of healthy leaves.

It is proposed to deal with several of these midges as opportunity arises. The button top midge (*Rhabdophaga heterobia* H.Lw.) has already received attention[1]. In this paper it is intended to give an account of the bionomics of the bat willow gall midge (*Rhabdophaga terminalis* H.Lw.).

I should like to state here how much I am indebted to Mr H. P. Hutchinson, the willow research officer at Long Ashton Research Station, for advice and providing sets of various species of willow; to Dr Imms who has supervised and discussed the work; and to all those whose knowledge of field conditions has been placed at my disposal, especially Mr Roebuck of the Midland Agricultural College, and Mr N. B. Warner Bromley.

2. DESCRIPTION OF ADULT AND DISTRIBUTION.

Rhabdophaga terminalis was first described by H. Loew (1850) as *Cecidomyia terminalis*. Later writers placed it in the genus *Perrisia* (*Dasyneura*), but now it is usually considered as belonging definitely to the genus *Rhabdophaga* Westw.

R. terminalis is typical of this genus, all of whose members bear a very close resemblance to species of the genus *Dasyneura* except that in *Rhabdophaga* the third vein joins the wing margin near or at the apex of the wing. The antennae consist of two basal segments and from 12 to 16 flagellar segments in the male and 11 to 15 in the females. The palps are composed of four segments, the fourth being the longest. The general size of the midge varies considerably, the body length being from about 1 mm. to slightly over 2 mm. In the female the abdomen is bright red until the eggs have been deposited, when it assumes a dark brown appearance similar to that of the male.

Until further studies of allied species have been made, it is not considered possible to identify this species on morphological characters alone, biological ones being essential.

The bat willow midge is recorded from western and central Europe, Denmark, Italy and England. In the last named country it has been found in Kent (Zimmermann, 1907), Wiltshire, and Suffolk and Norfolk. It is probably widely spread throughout the country. While not occurring in every commercial willow bed, in places where it does occur it is very liable to be exceedingly abundant.

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[1] Barnes, H. F., "Button Top" of Basket Willows, *Journ. Min. Agric.* April, 1929, pp. 65–71; On the Resistance of Basket Willows to Button Gall Formation, *Ann. Appl. Biol.* XVII, 1930, pp. 638–40; Further results of an investigation into the Resistance of Basket Willows to Button Gall Formation, *loc. cit.* XVIII, 1931, pp. 75–82.

3. BIONOMICS.

(*a*) *Emergence, mating, sex ratio and variation in adult.*

Emergence of the adults takes place at a definite time of the day; starting slightly before 8 a.m. (standard time), the maximum emergence of the males occurs about 10 a.m. and that of the females about noon, while very few individuals emerge after 5 p.m. (Barnes, 1930).

The males remain hovering round the soil from which emergence is taking place, awaiting the females. Fertilisation takes place immediately and without ceremony, coition lasting between 30 and 90 secs. One male will impregnate several females. Mating appears to be dependent on some chemotropic factor, as males have been observed hovering furiously round a position, *e.g.* leaf tip, which a virgin female has just left and have taken several minutes to find her in a new position. In the same way a male has been seen attempting to mate with a squashed virgin female when in reality there was only a shapeless mass remaining[1]. Various experiments have been conducted to see whether this species will mate with the button top midge (*R. heterobia*) and the leaf-curling pear midge (*D. pyri* Bouché). Negative results have been obtained in every case, no excitement of the males, no protrusion of the ovipositor by the females and no mating taking place.

The sex ratio at the time of emergence is rather interesting. It has been found previously (Barnes, 1931 *a*), that individuals reared from larvae collected in the field from golden willow (*S. alba* var. *vitellina*) gave a ratio of about 30 : 70, while individuals obtained in a similar manner from adjacent rows of bat willow (*S. coerulea*) gave ratios of 57 : 43, 70 : 30 and 16 : 84. These types of sex ratio were confirmed by breeding experiments under cold greenhouse conditions. Midges were reared in seven pots of *S. alba* var. *vitellina* and the sex ratio of the total midges bred was 16 : 84; on the other hand, using *S. coerulea* as the host plant in a similar number of pots, the figure of 54 : 46 was obtained. A possible reason for this wide range is the occurrence of unisexual progenies. It was found that in certain cases, where only a few females were used in setting up experiments, the subsequent generation consisted entirely of one sex. Allowing for the fact that it is quite possible that some of the females did not oviposit, it is highly probable that this indicates the phenomenon of unisexual families. The following figures are given in support of this hypothesis:

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[1] Occasionally in mating experiments with this species, when several males have been present in the company of virgin females, homosexuality has been observed, but in every case the offending male has subsequently died, probably as a result of some misplacement. This would prevent the possibility of such a tendency becoming inherited.

Cage A.	7 ♀♀	produced	0 ♂♂,	117 ♀♀
„ B.	*x* ♀♀	„	0 ♂♂,	63 ♀♀
„ C.	5 ♀♀	„	0 ♂♂,	70 ♀♀
„ D.	25 ♀♀	„	29 ♂♂,	0 ♀♀

It was found previously (Barnes, 1931 *b*) in another species of gall midge, *Thomasiniana oculiperda* Rübs., that this type of result correctly indicated the occurrence of unisexual families.

The adults of the bat willow midge vary considerably in size (Plate XII, figs. 1 and 2) according to the quantity of food available to the larvae (Barnes, 1932). Individuals may be from about 1 mm. to just over 2 mm. in body length. Besides this, the number of antennal segments may be from 2 + 11 in small females to 2 + 15 in large ones and from 2 + 12 in small under-nourished males to 2 + 16 in well-nourished ones.

An unusually coloured female was reared in 1928, the normal red colour of the abdomen being entirely absent and replaced by a whitish colour (Cecid. 1274).

(*b*) *Oviposition, types of gall and damage.*

Oviposition takes place a few minutes after mating. The eggs are laid in the terminal buds, being pushed in between the folds of the unopened leaves. Occasionally they are placed at the extreme base of lateral buds or at the base of the petioles. They are bright shining red in colour and the larvae hatch within 8 days or so, according to weather conditions.

The normal type of damage is shown in Plate XII, fig. 3 and Plate XIII, fig. 4. The terminal leaves remain in a curled and crinkled state instead of unfolding naturally. The gall is at first reddish in colour, but when the larvae have finished feeding it turns black and dries up. This holds good whether the pupae are in the gall or not. Occasionally one may find blister-like galls, as shown in Plate XIII, fig. 5, along the mid-vein of the leaf. This type occurs when, owing to adverse weather conditions, the development of the eggs is retarded to a greater degree than the growth of the plant. In such cases the leaves on which the eggs have been laid in the terminal bud develop in a normal manner, but the larvae hatch after a longer period than usual and not until after terminal growth of the plant has left these leaves down the stem, some distance from the growing point. The same type of gall results when the eggs are laid at the base of lateral buds and petioles. Usually the larvae hatch very quickly and produce the crinkled appearance of the leaves on which they have been laid before any appreciable amount of plant growth has taken place. As soon as the galls start developing terminal growth comes to an end.

Another position where the larvae may be found is in the galls of *Rhabdophaga rosaria* H.Lw., the large rosette-like galls which occur on various species of hedgerow *Salix* as well as on bat willow. Here the larvae live as inquilines. Individuals of the bat willow gall midge have been reared from galls of this species and have been found to interbreed quite normally with those from the usual type of gall. F. Loew (1875) states that the larvae of *R. heterobia* live occasionally as inquilines of *R. rosaria*. But one must now consider an error was made in the identification of *R. heterobia* and that he was dealing with the bat willow midge, *R. terminalis*. Individuals reared from larvae living as inquilines of *R. rosaria* will mate and interbreed with *R. terminalis* on the one hand; but on the other, *R. heterobia* will not mate at all with *R. terminalis*.

The damage done by this midge is very similar to that caused by the button top midge (*R. heterobia*). Terminal growth of the attacked shoots is stopped and side shoots develop. This renders the rods practically useless. In the case of attacks on golden willow (*S. alba* var. *vitellina*) the rods are spoilt from the basket-making point of view. In the case of attacks on bat willow (*S. coerulea*) it is much more serious. The bat willow is grown for cricket bats and it is usual to plant sets of 8–12 ft. long and slightly thicker than broomsticks. Once these sets are planted they should not be moved, and the trees should be clear of branches at least 15 ft. from the ground. A method of growing these sets is to allow rods from a stub to continue growing for a period of 2–3 years before cutting and then transplant the sets into their permanent positions. It is absolutely essential that the sets be free from side shoots. The bat willow midge sometimes causes great havoc in places where these sets are being grown and owing to their value the monetary loss is very considerable.

The bat willow midges living as inquilines in the galls of *R. rosaria* cannot be considered to be directly injurious, as the larvae of *R. rosaria* are the cause of the side branching in these cases. But, since *R. rosaria* occurs on indigenous hedgerow species of *Salix*, the presence of the inquilines is an additional reservoir of infection which can easily be overlooked.

(*c*) *Host plants with results of preference and immunity trials.*

Previous workers have recorded this midge from several species of *Salix*, including *S. alba*, *fragilis*, *amygdalina*, *hastata*, *pentandra*, *purpurea*, *viridis* and *repens* (Houard, 1908; Kieffer, 1901). Such records must be considered unproven until biological work has shown that this midge will attack such a wide range of *Salix* species.

Investigations carried out at Rothamsted have shown that there are definite host plant preferences and immunities. The experiments were carried out in the same manner as those described in the trials of the resistance of basket willows to the button top midge (Barnes, 1931 *c*).

In the field this species has only been found attacking the bat willow (*S. coerulea*) and a golden willow, *S. alba* var. *vitellina*.

Midges, when given a choice of ovipositing on bat willow (*S. coerulea*) or *S. alba* var. *vitellina*, invariably chose the bat willow[1]. This was true whether the midges had been reared on *S. coerulea* or *S. alba* var. *vitellina* for one or two generations previously. There was no inclination for those reared on the latter to oviposit on *S. alba* var. *vitellina*. Perhaps if they had been reared for many generations on the one host plant they might prefer to lay on it rather than on *S. coerulea*.

If the midges had no choice but had to lay on *S. alba* var. *vitellina*, they did so quite readily and subsequently the normal development took place.

The following varieties and species withstood all attacks, both in preference and immunity trials: Black Maul (*S. triandra*), Long Skin (*S. viminalis*) and Dicky Meadow (*S. purpurea*).

S. alba var. *cardinalis* was subjected to preference trials only, and proved to be immune in the presence of bat willow (*S. coerulea*), *S. alba* var. *vitellina* and Black Maul (*S. triandra*). This does not prove that it is immune in reality, since *vitellina* was not attacked in these circumstances. But it does show that it is not more attractive for oviposition than *S. coerulea*.

(*d*) *Life cycle.*

The galls of the last brood of the year fall to the ground in the autumn with the leaves, carrying with them any larvae that have not previously dropped to the soil. The larvae remain in the surface layers until the following spring. Pupation of this generation takes place in the soil.

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[1] For example, in one instance, 1931, in a cage containing 9 bat willow and 10 *vitellina* plants, 8 out of the 9 bat plants were attacked but none of the *vitellina* ones. As the plants were randomised, the ovipositing females must have flown up and down the cage choosing the bat plants in distinct preference to the *vitellina*.

In early May the first flight of the adults takes place, and subsequently further generations are on the wing at about monthly intervals throughout the summer until September. Each flight, except the first which may go on for as long as a month, usually lasts about a week or 10 days. The larvae of the summer broods pupate in the galls as a rule but can do so equally well in the ground if they are disturbed or knocked out of the gall either by birds or overcrowding. Up to 40 larvae have on occasion been found in a single gall. The duration of the pupal stage is about a week.

Under unheated greenhouse conditions in 1931 four generations were completed during the summer, thus there were five flights during the year. Only partial emergences of the third and fourth generation, and none of the fifth, took place the same year. The actual dates of oviposition and emergence, together with the duration of the generation can be seen in Table I.

Table I.

Showing dates of oviposition, flights of adults and time taken to complete the life cycle under unheated greenhouse conditions, 1931.

Date of oviposition	Dates of flight of midges	Time in days taken to complete life cycle
May 10–11	June 13–19	34
„ 12	„ 13–15	32
„ 13	„ 13–19	31
June 13	July 6–19	23
„ 14	„ 6–11	22
„ 13–14	„ 9–12	26
„ 13–14	„ 8–18	25
July 6	Aug. 1–6	26
„ 6–11	„ 1–6	26
„ 7–8	„ 2–9	26
„ 8	„ 2–11	25
„ 7	„ 5–16	29
„ 7	„ 4	28
Aug. 4–5	Sept. 1–10	28
„ 2–5	Aug. 30–Sept. 10	28
„ 3–4	„ 31– „ 10	28

The midges were reared both on bat and golden willow. It is interesting to note that the cycle took 31–34 days to complete in May-June, but less (22–26) in June-July, and slightly longer again in July-August (25–29) and August–September (28). In a previous year in the same greenhouse there was one flight less.

Observations in the field suggest that there are usually four flights during the year, that in more favourable seasons it is probable there is at least one more and in unfavourable ones probably one less. Considerable overlapping takes place, however, it being possible to find adults and larvae in all stages almost at any time between late May and the end of August.

It is also considered as highly probable, if not certain, that some individuals of the antipenultimate and penultimate generations of the year remain in the soil as larvae, not emerging until the following spring. It has been proved that they do this when reared in pots and under unheated greenhouse conditions, and also that fully fed larvae of the penultimate generation of the season obtained from the field and kept in pots in an outdoor insectary do likewise.

4. CONTROL.

There is no real control yet known for this midge. Certain Hymenopterous Chalcid parasites are usually present but do not appear to breed fast enough to exert any very great control. The bug *Anthocoris nemorum* L. is frequently found sucking either adults as they are emerging or the larvae in the galls. Tits also eat considerable numbers of the larvae, picking them out of the galls. In spite of these natural enemies, this midge seems to multiply very rapidly, and by the late summer in some years there must be literally millions in some willow beds. Fortunately this midge, although liable to appear in epidemic proportions on occasion, is often noteworthy because of its greatly diminished numbers. Some factor or factors in the weather complex seem to have a controlling influence over it. What this is still remains to be discovered; it may have a direct effect on the midge itself or it may have differential effects on the host insect and its parasites and so be acting indirectly on the midge.

As to artificial control measures there is unfortunately little to say. Handpicking the galls can be suggested where school children can be employed cheaply. It is suggested that cultivating the soil between the stubs very thoroughly in March and April might diminish the numbers of the first flight in May very considerably. Massee (1931) has reported that he found cultivation very effective in the case of the pear midge (*Contarinia pyrivora*). The essential feature is that the cultivator must be taken across the rows, along the rows and also diagonally across them. It should be done once a week for about 4 or 5 weeks with a view to exposing the larvae. Hand hoeing round the stubs must accompany

any such cultivation. In the case of the pear midge the cultivation was done immediately the larvae had descended to the soil about June 10th, but this midge is only single brooded. The cultivation in March and April is recommended in the case of the bat willow midge because it is about this time that all the larvae are in the soil. Throughout the summer there are larvae up in the galls. It might be effective to do the cultivation in the late autumn if it were a rather cold one. But the east winds, general lack of insect food for birds, and the possibility of frosty weather in March make this period seem more advantageous. The cost of such extensive cultivation would be amply compensated for by the value of clean cricket bat sets; in the case of golden willows it might not be economical.

All hedgerow *Salix* bushes should be exterminated, as they may be acting as reservoirs of this midge ready to re-infest the fields.

5. SUMMARY.

1. The bionomics of the bat willow gall midge (*Rhabdophaga terminalis* H.Lw.), which does serious damage to certain willows grown for basket making and the cricket bat willow grown for sets, have been studied.

2. The midge exhibits a distinct host-plant preference, choosing the bat willow (*S. coerulea*) when possible. But it also breeds readily on a golden willow, *S. alba* var. *vitellina*. It will not attack Black Maul (*S. triandra*), Long Skin (*S. viminalis*) and Dicky Meadow (*S. purpurea*).

3. It is shown to be a species which sometimes occurs in epidemic numbers. Intensive cultivation is suggested as a control.

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EXPLANATION OF PLATES XII, XIII.

PLATE XII.

Fig. 1. Small female *R. terminalis*, × 20.

Fig. 2. Large female *R. terminalis*, × 15.

Fig. 3. Typical gall of *R. terminalis* in dried up condition denoting the presence of pupae, showing damage by *Phyllodecta vitellinae* on the outer leaves.

PLATE XIII.

Fig. 4. Four typical galls containing larvae on *S. alba* var. *vitellina.*

Fig. 5. Alternate type of gall, when the terminal bud has grown away from the attack, or when the eggs have been laid at the base of lateral buds and petioles instead of on the terminal bud.

(*Received November* 25*th*, 1931.)

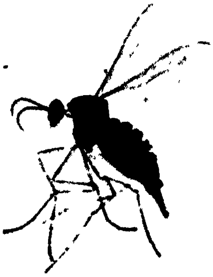

Fig 1.

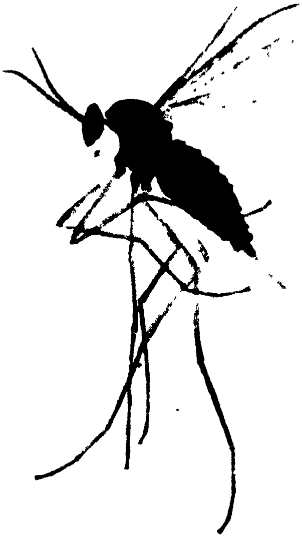

Fig. 2.

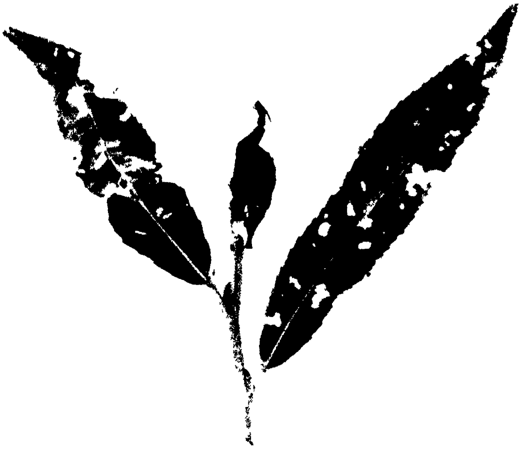

Fig 3.

BARNES.—On the Gall Midges Injurious to the Cultivation of Willows (pp. 243–252).

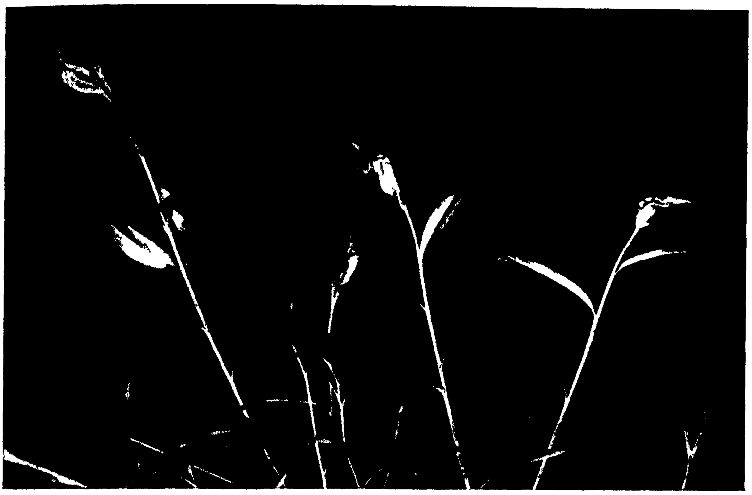

Fig. 4.

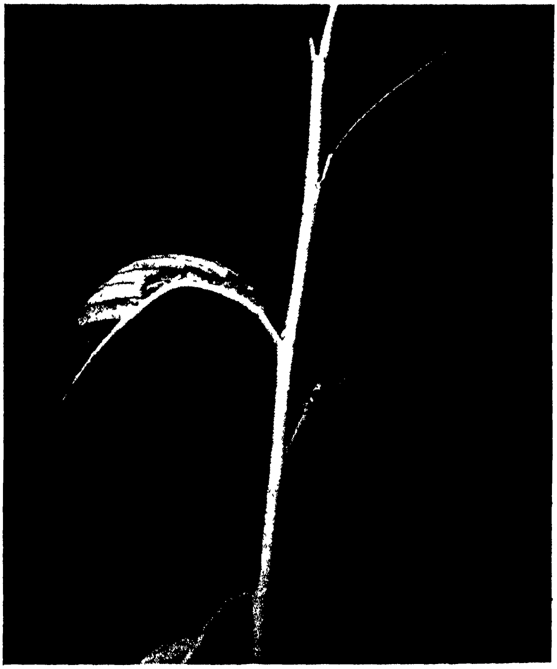

Fig. 5.

BARNES.—On the Gall Midges Injurious to the Cultivation of Willows (pp. 243–252).

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A Study of the Segmentation of the Antennæ in Gall Midges (Cecidomyidæ).

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(Plate I.*)

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1. INTRODUCTION.

The number of antennal segments in the Cecidomyidæ differs in the various subfamilies and genera. Thus, according to Felt (1925), "the Asphondyliariæ have almost invariably fourteen antennal segments." Further, "the relative stability in the number of antennal segments characteristic of the Itonididinariæ (Cecidomyiariæ) and the Asphondyliariæ presents a marked contrast to that found among the Dasyneuriariæ and the Oligotrophiariæ, there being a variation in the latter two groups of from nine to twenty-five or twenty-six segments."

He goes on to state "There may be in this group (Lasiopteriariæ) a variation between the sexes of three or four to seven in the number of antennal segments, and it is not unusual to find a difference of two, or possibly three, among individuals of the same sex."

Finally, Felt claims "There appears to be some connection between the size of the insect and the number of antennal segments. The larger forms of *Rhopalomyia* Rubs., *Rhabdophaga* Westw., and *Hormomyia* H. Lw. have the greatest number of antennal segments."

Such statements as these, based on very wide experience in the Cecidomyidæ, are undoubtedly true, and are supported by other specialists in this family.

It seems curious that no one, with a solitary exception, appears to have made a study of this variation in segmentation apart from casual observations. The exception is Wehrle (1924), who made a study of the segmentation of the antennæ of the clover-seed midge (*Dasyneura leguminicola* Lintner). Using only 115 individuals (80 females and 35 males), he drew several conclusions. Firstly, he found that the number of segments in the antennæ varied from thirteen to seventeen, the commonest number for both sexes being sixteen.

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* For explanation of the Plate, see p. 334

It will be seen that it is exceedingly doubtful whether this latter statement, that the commonest number is the same for both sexes, can be upheld generally in view of the results to be expressed in this paper. Secondly, he found that the two antennæ of an individual specimen may have either an equal or an unequal number of segments. Finally, Wehrle states: "Certain individuals occur in both sexes in which there is a certain lack of definiteness in the segmentation of the antennæ. The distal segment may be slightly constricted, indicating the beginning of the differentiation of another segment. Some of the segments posterior to the distal segments may fuse or be grown together. This is due to the imperfect differentiation of the segments." It may be as well to point out now that the present writer agrees with these findings except as stated above.

While studying the Cecidomyidæ this variation in segmentation of the antennæ became very apparent, and, having ample material, it was decided to inquire into the matter more closely. Slightly over 14,300 individuals were examined during the course of this study.

2. Species used in the Study and Method.

The material used consisted of large numbers of bred specimens of *Rhabdophaga heterobia* H. Lw., *R. terminalis* H. Lw., *Dasyneura alopecuri* Reuter, *D. pyri* Bouché, *D. arabis* Barnes, *Colomyia hordei* Barnes, *Contarinia tritici* Kirby, *C. merceri* Barnes, *C. tragopogonis* (Kieffer) Barnes, *Sitodiplosis mosellana* Géhin, *Stenodiplosis geniculati* Reuter, *Lestodiplosis achilleæ* Barnes, *Thomasiniana theobaldi* Barnes, and *T. oculiperda* Rübs.

One hundred specimens of one sex were taken to be a sample except where fewer individuals were available. This sample was examined and the results first tabulated into a scheme such as is partly figured below (Table I.).

Table I.—Showing first stage in separation of individuals into groups possessing different numbers of antennal segments.

Type and number of antennal segments.	Number of individuals.
2+16+big	1
2 +17	3
2 +15+big	16
$2+\left\{\begin{matrix}17\\15+\text{big}\end{matrix}\right.$	1
$2+\left\{\begin{matrix}17\\16\end{matrix}\right.$	1
2+ 16	35
2+ 14+big	8
$2+\left\{\begin{matrix}16\\14+\text{big}\end{matrix}\right.$	1

This scheme was made to cover the entire range of variation in the sample being considered, in this case 2+18 to 2+13. The two basal segments forming the scape are indicated by the 2, while the number of the flagellar segments are represented by the subsequent figure. Thus an individual with 18 flagellar segments would be classed as 2+18. Further, it was found that the terminal segment was not always clearly indicated, and was often twice as large as the preceding one and only partially demarcated. In such a case the individual would be classified as 2+15+ big or 2+14+big, etc. In some cases one antenna would have one number of segments while the other would have another number. Here the individual would be recorded as $2+\begin{cases}17\\16\end{cases}$ when all the segments were differentiated, or $2+\begin{cases}17\\14+\text{big}\end{cases}$ when the terminal segment was not differentiated on one antenna while differentiated on the other. Similarly, the two antennæ of an individual might consist of the same number of segments, but in the case of one antenna clearly differentiated segments (say 2+16) and in the case of the other antenna the terminal segments not properly defined (say 2+14+big); in such cases the individual would go into the category $2+\begin{cases}16\\14+\text{big}\end{cases}$. Such was the primary division into groups.

The next step was to collect together the groups which contained the sane number of antennal segments. Thus the 2+17 group, the 2+15+big, and the $2+\begin{cases}17\\15+\text{big}\end{cases}$ group would be classed as 2+17. This procedure would give a scheme as in Table II. In this table two samples of *R. heterobia* males, one bred in 1928 and the other in 1929, are being compared. In some cases three samples of 100 individuals were used to see the extent of variation between the separate hundreds †. An average was then written in, taking care to denote the extreme limits 2+18 and 2+13, even if there was only a single individual in either of these classes. Part of the second hundred in the 1928 sample is a continuation of Table I. It will be seen that the agreement between the first, second, and third samples of 100 individuals is quite close.

TABLE II.—Showing second stage in separation of individuals into groups possessing different numbers of antennal segments.

Number of antennal segments.	1928. Number of individuals				1929. Number of individuals.			
	1st 100	2nd 100.	3rd 100.	Average.	1st 100.	2nd 100	3rd 100	Average
2+ 18	—	1*	—	1				
$2+\begin{cases}18\\17\end{cases}$								
2+ 17	22	20*	16	19	4	3	6	4
$2+\begin{cases}17\\16\end{cases}$	—	1*	4	2	2	1	1	1
2+ 16	42	44*	52	46	45	46	40	44
$2+\begin{cases}16\\15\end{cases}$	3	2	1	2	—	—	1	1
2+ 15	27	28	25	26	33	36	37	35
$2+\begin{cases}15\\14\end{cases}$	—	—	—	—	—	1	1	1
2+ 14	4	3	2	3	14	12	10	12
$2+\begin{cases}14\\13\end{cases}$								
2+ 13	2	1	—	1	2	1	4	2

* Denotes the part of the figure carried on from Table I.

† Similarly, in one case the sample was divided into three smaller samples of 25 individuals. The result of examining these samples was as follows —2+17 (2.13.6.2.1) 13, 2+17 (2.14.5.3.0) 13, and 2+17 (2.13.6.1.2) 13.

Finally, the third stage was to write out the *antennal formula*, neglecting the intermediate antennal numbers; thus the 1928 sample has the formula 2+18 (1.19.46.26.3.1) 13, while the 1929 sample has the formula 2+17 (4.44.35.12.2) 13. It will be noticed that the numbers inside the brackets in the 1928 formula add up to 96 and those in the 1929 sample add up to 97. This indicates that four and three individuals respectively out of 100 have an intermediate number of antennal segments, such as $2+\left\{\begin{matrix}17\\16\end{matrix}\right.$ or $2+\left\{\begin{matrix}16\\15\end{matrix}\right.$. The numbers inside the brackets represent the number of individuals in the different classes; for example, in the 1929 formula they mean that 4 individuals have 2+17 segments, 44 have 2+16, 35 have 2+15, 12 have 2+14, and 2 have 2+13.

Having obtained such a formula, it is possible to compare within the species the antennal segments of one brood with another, one sex with the other, etc., to compare one species with another; to fix the range of variation and to indicate the frequency with which one number of antennal segments occurs as compared with another.

This is the method that was used throughout in obtaining the formulæ. The number in brackets before the formula indicates the number of separate hundreds used in obtaining the formula. Thus (3.0) 2+18 (1.19.46.26.3.1) 13 means that the formula is the average of three separate hundreds. Where less than 100 and more than 50 were used, the figure in brackets still indicates how many individuals were used—thus (0.87) and (0.5) mean 87 and 50 individuals respectively; the numbers inside the brackets were multiplied up to represent 100, less, of course, all intermediates. Where the numbers used were less than 50 there is an asterisk before the formula, and the numbers inside the brackets of the formula itself represent the actual numbers examined, less the intermediates.

3. CONSTANT SEGMENTATION.

Species in five genera were examined, and in each case the number of antennal segments was constant both for the genus and the species. For example, in the genus *Contarinia* three species—*tritici* Kirby, *merceri* Barnes, and *tragopogonis* (Kieffer) Barnes—were included in the study. Two hundred specimens of each sex of *C. tritici* gave, without exception, 2+12 (100) as the antennal formula. A similar number of male and twice the number of female *C. merceri* gave the same result, 2+12 (100), while one hundred of each sex of *C. tragopogonis* did the same, 2+12 (100).

Stenodiplosis geniculati Reuter (255 ♂♂, 234 ♀♀) in every case gave the same formula, 2+12 (100). *Sitodiplosis mosellana* Géhin did likewise, 2+12 (100). Twenty male and twenty-five female *Lestodiplosis achilleæ* Barnes returned the formula 2+12 (100). *Thomasiniana theobaldi* * Barnes and *T. oculiperda* Rübs. always gave 2+12.

It must be mentioned that these samples of midges were chosen for difference in locality, season, size of individual and of host-plant. None of these factors altered the antennal formula. For instance, the largest individuals of *C. tritici*, as well as the smallest of either sex, always gave 2+12, and *T. theobaldi*, whether reared on "Lloyd George" or "Bath's Perfection" raspberry, remained constant as regards antennal numbers.

This absolute degree of constancy among these species and genera, in spite of differences in food-supply, is remarkable in view of what occurs in other genera and species.

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* 125 males and 151 females of this species and 100 males and 200 females of *T. oculiperda* were used

4. VARIABLE SEGMENTATION.

In species belonging to three genera—*Rhabdophaga*, *Dasyneura*, and *Colomyia*—the number of antennal segments has been found to be extremely variable. For example, in *R. heterobia* H. Lw. the number of segments in the male was found to vary from 2+18 to 2+13, with the majority of individuals having 2+16; in the female it varied from 2+17 to 2+12, with the majority having 2+15. Other species gave similar results (Table III.).

TABLE III.—Species of midges whose antennæ have a variable number of antennal segments.

Species.	Number of antennal segments.					
	Maximum.		Minimum.		Most individuals.	
	♂.	♀.	♂.	♀.	♂.	♀.
R. terminalis H. Lw.	2+ 16	2+15	2+12	2+ 11	2+14	2+13
D. alopecuri Reuter	2+ 17	2+15	2+11	2+ 10	2+15	2+14
D. arabis Barnes	2+ 13	2+13	2+12	2+ 11	2+13	2+12
D. pyri Bouché	$2+\left\{\begin{matrix}15\\14\end{matrix}\right.$	2+14	2+10	$2+\left\{\begin{matrix}11\\10\end{matrix}\right.$	2+14 and 2+13	2+13
C. hordei Barnes	2+ 24	2+27	2+16	2+ 19	2+18 and 2+19	2+23 and 2+24

Samples of these species lead one to the conclusion that in the genera *Dasyneura* and *Rhabdophaga* the males, as a rule, have one more segment than the females ; but in other genera, such as *Colomyia*, the female has more than the male. Kieffer (1913) states that the females of *Lasioptera* have more than the males.

Since such variation was noticed, it was decided to examine material bred under different conditions, *e. g.*, those of season, locality, type of gall, host-plant, and climate, in order to discover whether; first, these ranges of variation were constant within the species in the first place, and second, whether the numbers within the antennal formula would be altered. Before passing to details it may be stated that the range of variation in a species was found to be usually constant, but that there was frequently a movement of numbers within the formula.

(a) *Effect of Season.*

In order to determine whether the antennal formula for any one species remains constant over a period of seasons, samples of midges, obtained from one locality over a period of years, were examined.

Thus samples were examined of *R. heterobia* which emerged during 1928, 1929, 1930, and 1931, larvæ in each case having been collected in the button-type of gall at Syston, Leicestershire, the previous year. The following formulæ were obtained :—

	♂♂.
1928	(3.0) 2+18 (1.19 46.26.3.1) 13
1929	(3.0) 2+17 (4.44.35.12.2) 13
1930	(1.0) 2+18 (1.19.53 25.2) 14
1931	(1.0) 2+17 (15.59.22.4) 14

♀♀.

1928......	(3.0) 2+17 (1.22.54.20.2) 13
1929......	(2.0) 2+16 (7.37.32.18.5) 12
1930......	(1.0) 2+16 (2.39.47.11.1) 12
1931	(1.0) 2+16 (16.56.23.5) 13

In each case the same brood (overwintering as fully-fed larvæ) was used.

In the case of *D. alopecuri* the source of supply was near Aberdeen, and again the same brood was used :—

♂♂.

1928.....	(3.0) 2+16 (7.60.28.3.0.1) 11
1929......	(2.0) 2+17 (1.26.66.7) 14
1930... ..	(1.0) 2+17 (1.41.52.3.2) 13
1931	(1.0) 2+16 (22.72.4.1) 13

♀♀.

1928 .. .	(3.0) 2+15 (3.48.38.9) 12
1929 . .	(2.0) 2+15 (17.76.7) 13
1930......	(1.0) 2+15 (44.41.12.3) 12
1931	(1.0) 2+15 (31.63.6) 13

It can be seen from the above figures that samples of these species of midge have different antennal formulæ in different years.

(b) *Effect of Locality.*

Samples of the larvæ of a single-brooded midge, *D. alopecuri*, were obtained in 1927 (the midges emerging in 1928) from various localities. An examination of the adults gave the following results :—

♂♂.

Rutlandshire	(1 0) 2+15 (65.33.0.1) 12
Derbyshire	(1.0) 2+16 (8.87.2.1) 13
Leicestershire	(1.0) 2+16 (1.70.25.2) 13
Lincolnshire	(1.0) 2+16 (5.76.14.1) 13
Yorkshire	(1.0) 2+15 (43.46.7.1) 12
Nottinghamshire ...	(1.0) 2+16 (2.60.33.2) 13
Co. Tyrone	(1·0) 2+15 (57.37.4.1) 12
Co. Dublin	(1.0) 2+16 (8.71.16.2) 13
Co. Antrim (1)	(1.0) 2+16 (4.61.28.3.1) 12
Co. Antrim (2)	(3.0) 2+16 (3.72.20.1.1) 12
Aberdeen	(3.0) 2+16 (7.60.28.3.0.1) 11

♀♀.

Rutlandshire	(1.0) 2+15 (2.56.31.5) 12
Derbyshire	(1.0) 2+15 (5.81.11) 13
Leicestershire	(1.0) 2+15 (4.53.35.4.1) 11
Lincolnshire	(1.0) 2+15 (4.61.29.3) 12
Yorkshire	(1.0) 2+15 (3.24.52.18.3) 11
Nottinghamshire ...	(1.0) 2+14 (50.40.8) 12
Co. Tyrone...........	(1.0) 2+15 (2.46.39.8.3.1) 10
Co. Dublin...........	(1.0) 2+15 (5.70.18.6) 12
Co. Antrim (1)	(1.0) 2+15 (3.51.37.7) 12
Co. Antrim (2)	(3.0) 2+15 (11.58.24.5) 12
Aberdeen	(3.0) 2+15 (3.48.33.9) 12

It can be seen from the above figures that samples of a single species of midge of the same brood and the same year, but from various localities, will give different antennal formulæ.

(c) *Effect of Type of Gall.*

The larvæ of *R. heterobia* form three types of galls according to the position on the plant in which the eggs are laid. Two of these types have been taken into consideration—the " button " gall, which is formed on the terminal growing point or bud, and the " bud " type, which is formed on the lateral buds of a shoot. The following are the formulæ for the midges emerging in 1929 from these two kinds of gall from the same locality (Syston) :—

	♂♂.
Button type of gall	(3.0) 2+17 (4.44.35.12.2) 13
Bud „ „ ..	(2.0) 2+17 (10.64.19.3) 14
	♀♀.
Button type of gall	(2.0) 2+16 (7.37.32.18.5) 12
Bud „ „	(2.0) 2+16 (3.58 32.4.1) 12

It would appear, therefore, that midges from the " bud " gall are inclined to have more antennal segments than those from " button " galls.

(d) *Effect of Change of Host-plant and Captivity.*

The midge *R. terminalis* lives on the Golden variety of willow, *Salix alba* var. *vitellina*, and on Bat willow, *S. cœrulea*. The fully-grown larvæ were collected in Suffolk from adjacent rows of the two varieties of willow. The following are the formulæ of midges of the same brood and year [†] from the two host-plants :—

	♂♂.	
Sample.	Host-plant	Formulæ.
A	Golden.	(2.0) 2+15 (5.35.50.7) 12
A	Bat.	(2.0) 2+16 (1.16.57.22.1) 12
B	Golden.	(0.5) 2+15 (20.46.20.2) 12
B	Bat.	(0.87) 2+15 (47.43.6) 13
	♀♀.	
A	Golden.	(2.0) 2+14 (8 43.42.6) 11
A	Bat.	(2.0) 2+15 (2.12.62.21.1) 11
B	Golden.	(1.0) 2+14 (25.45.30) 12
B	Bat.	(1.0) 2+15 (2.66.29.3) 12

It would appear from these figures that the midges living on Bat willow are inclined to have more antennal segments than those living on Golden willow. But some midges which had been reared from Bat variety were, in 1929, allowed to oviposit on Golden willow. In this case their offspring were all exceptionally large. Twenty-five females were examined, and gave the following antennal formula :—*2+15 (19.6) 14. Similarly, in 1931 midges reared from Golden willow were given the choice of ovipositing on Golden and Bat varieties, but only laid on Bat willow. The resulting generation of midges were likewise very large individually, and five individuals examined all had 2+15 antennal segments.

[*] Where an asterisk is printed at the beginning of the formula, the actual numbers of individuals examined are to be found within the brackets of the formula.

[†] Sample A=August brood 1928, sample B=June brood 1929.

It seems, therefore, that midges from either host-plant can, under certain conditions, have the higher range of antennal segments.

The effect of captivity on the antennal formula is interesting. The following are the figures for *D. arabis*, which had been continuously bred in captivity :—

		♂♂.	
1st year	1st brood in captivity.		—
	4th	,, ,,	(1.0) 2 +13 (90.6) 12
2nd year	5th	,, ,,	(1.0) 2+13 (81.18) 12
	6th	,, ,,	(1.0) 2+13 (82.18) 12
3rd year.	9th	,, ,,	(1.0) 2+13 (71.27) 12
		♀♀.	
1st year	1st brood in captivity.		(1.0) 2+12 (98 2) 11
	4th	,, ,,	(1.0) 2+12 (94.6) 11
2nd year	5th	,, ,,	(1 0) 2+13 (4.78.18) 11
	6th	,, ,,	(1.0) 2+12 (70.18) 11
3rd year	9th	,, ,,	(1.0) 2+12 (52 44) 11

It will be seen that the longer the midges have been in captivity the more the number of antennal segments is reduced. This may be due to the fact that the natural health of midge and host-plant is very hard to maintain under artificial conditions. As well as the number of antennal segments being reduced, the general size of the individuals was observed to decrease.

(e) *Effect of Climate.*

In order to see whether variety of climate has any effect on the number of antennal segments, material which had been bred under different conditions of temperature was examined. Two species of midge, *D. alopecuri* and *R. heterobia*, were used. The control (C.) in each case was material bred under outside insectary conditions. Some material (E.C.) was subjected to extra cold in the form of a temperature of −12° C. for a period of 28 days, then it was placed in the insectary until emergence took place. Other material (H.) was kept in a heated greenhouse and allowed to emerge during the winter. The following formulæ were obtained (Table IV.) :—

TABLE IV.—Effect of extra cold and extra heat.

Condition.	Species.	♂♂.	♀♀.
C.	*D. alopecuri.*	(2 0) 2+17 (1.26.66.7) 14	(2.0) 2+15 (17.76.7) 13
E.C.	,,	(2.0) 2+16 (32 62.4) 14	(2.0) 2+15 (27.65.8) 13
H.	,,	(2.0) 2+16 (36.59.1) 14	(2.0) 2+15 (43 56) 14
C.	*R. heterobia* A.	(3.0) 2+17 (4.44.35.12.2) 13	(2.0) 2+16 (7.37.32.18.5) 12
	,, B	(2.0) 2+17 (10.64.19.3) 14	(2.0) 2+16 (3.58.32.4.1) 12
E.C.	,, A.	(1.0) 2+17 (8.42.34.10.4) 13	(1.0) 2+16 (1.40.44.14) 13
	,, B.	(0.5) 2+17 (9 72.15) 15	(0.5) 2+16 (5.63.32) 14
H. .	,,	(0.75) 2+17 (8.53 23.8.4) 13	(1.0) 2+16 (15.48.29.8) 13
Control for H. .	,,	(1.0) 2+18 (1.19.53.25.2) 14	(1.0) 2+16 (2.39.47.11.1) 12

Both button (A in Table IV.) and bud galls (B.) were used in the case of *R. heterobia*, and the material subjected to extra heat (H.) belonged to a subsequent season to that used in the extra cold experiment—hence there was a different control. In the heat experiment only button galls were used.

It would appear that in the case of *D. alopecuri* the alteration in temperature affects the number of antennal segments, and that individuals bred under these conditions have more antennal segments than those under normal conditions. In the females this change is more pronounced than in the males. This is interesting in the light of previous work on the effect of temperature on the emergence of this species (Barnes, 1930). One of the conclusions reached was that non-lethal conditions affected females more than males, but that lethal conditions, *e. g.*, change in temperature, affected the males more than the females. Further, when midges were subjected to continuous heat and allowed to emerge under these conditions, during the winter the percentage emergence was 58·6±5 instead of 76·1±1·9 under normal conditions; in other words, there was a lethal effect on some of the midges. Now if the midges killed were the ones with the fewer numbers of antennal segments, one would expect that more of the survivors would have the larger numbers of antennal segments, which is exactly what is seen to happen. Yet this would not agree with the conclusion that lethal conditions affected males more than females, unless one assumes that females are more unstable as regards the number of antennal segments than males.

In the case of *R. heterobia* the effect of change of temperature on the number of antennal segments is much less; if there is an effect it is to increase the number of individuals possessing the largest numbers of antennal segments.

A further experiment with regard to the effect of light on midge emergence provided more material for this study. The following formulæ were obtained for *D. alopecuri* emerging under conditions of total darkness (D.). *R. heterobia* was not used in this light experiment.

Condition.	♂♂.	♀♀.
Normal light control	(2.0) 2+17 (1.26.66.7) 14	(2.0) 2+15 (17.76.7) 13
Total darkness (D.)	(2.0) 2+16 (18.79.2) 14	(2.0) 2+15 (36.64) 14

It was found previously (*loc. cit.*) that females were more affected by this change of condition than the males. The alteration in number of antennal segments towards the larger numbers again appears to be more pronounced in the females than in the males.

5. IS THE NUMBER OF ANTENNAL SEGMENTS INHERITED?

In order to answer this question satisfactorily it would be necessary to breed from parents of known antennal numbers. Sufficient time has not been available to make such a study possible, but some evidence is put forward to show that it is unlikely that the number of antennal segments is inherited.

An all-male family of *R. heterobia* which had only been in captivity for one generation gave the formula (0·64) 2+16 (56.42.2) 14, while an all-female family, also in captivity for only one generation, gave the formula (0·43) *2+16 (4.32.7) 14. Individuals from these two families were mated with the following results in two cases:—

Parents.	Formula of Generation 1.
♂ 2+16; ♀ 2+15 .	(0.1) *2+15 (3.6.1) 13 · an all-male family.
♂ unknown; ♀ 2+15 . .	(0.13) *2+14 (7.5.1) 12 · an all-female family.

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* Actual numbers.

The number of individuals is very small, but in each case the number of antennal segments is inclined to be distinctly smaller than that of the parents, and is exactly what one would expect if a second generation in captivity were to affect the number of antennal segments.

Other single families of *R. heterobia* have been examined, with the following results :—

	♂♂.
Family 32	(0.59) *2+17 (6.36.17) 15
„ 41	(0·36) *2+16 (19.17) 15
„ 44	(0.28) *2+16 (22.6) 15
„ 28	(0.13) *2+16 (7.6) 15

	♀♀.
Family 30	(0.7) *2+15 (52.18) 14
„ 18	(1 0) *2+16 (1.62.36.1) 13
„ 6	(0.39) *2+15 (14 22.3) 13
„ 35	(0.44) *2+15 (29.13.2) 13

Further a male *D. arabis* (2+13) was mated to six females and the resulting families of midges were examined with the following results :—

Parent.	Family.	
	♂♂.	♀♀.
1st ♀ 2+12	*2+13 (3)	*2+12 (19.3) 11
2nd ♀ $2+\left\{\begin{matrix}11\\12\end{matrix}\right.$	*2+13 (21.2) 12	*2+12 (3.1) 11
3rd ♀ 2+12	*2+13 (7.2) 12	*2+12 (2)
4th ♀ 2+12	*2+13 (16)	*2+12 (11.3) 11
5th ♀ 2+12	*2+13 (3.1) 12	*2+12 (7.18) 11
6th unknown	*2+13 (3.1) 12	*2+12 (5.7) 11

If this slight evidence is weighed with that in sections 4 and 6, it is apparent that, while there is reason enough to suppose that the number of antennal segments is affected by external environmental conditions, there are few, if any, indications in favour of inheritance.

6. NUMBER OF ANTENNAL SEGMENTS AND SIZE OF MIDGE.

It has been observed repeatedly that those midges emerging at the end of the emergence period of a sample are inclined to be smaller in size than those emerging earlier.

In order to test whether the antennal number of such smaller midges was different to that of the larger midges, one sample of a midge *R. terminalis* was used. The female midges emerging during the main period of emergence in this sample were placed day by day into a vial, while those appearing during the last week or so were placed in another. One hundred midges (A) out of the first 432 to emerge, and 50 (B) out of the last 67, were examined, with the following results :—

A	(1.0) 2+15 (2.66.29.3) 12
B	(0.5) 2+14 (12.30.46.12) 11

This would seem to be evidence that the midges which emerge late in the emergence period have a smaller number of antennal segments than those

---

* Actual numbers.

appearing earlier. But more interesting is the correlation between the size of midge and the number of antennal segments. These midges show a distinct difference in size as well as a corresponding difference in antennal numbers.

Again, while examining the samples it has frequently been the custom to place the midges with different numbers of antennal segments in separate vials. If these vials are arranged according to the number of antennal segments, say 2+15 to 2+11, and if the midges in them are then examined, it will be seen that the groupings might almost have been made according to the size of midge. In Pl. I. figs. 1–5 are photographs of five individuals of *R. terminalis* (♀) from a 2+11 vial, a 2+12 vial, a 2+13 vial, a 2+14 vial, and a 2+15 vial. It will at once be seen that the midges form an ascending scale in size from fig. 1 (2+11) to fig. 5 (2+15). This is further evidence of the correlation between the number of antennal segments and the size of the midge.

A similar correlation has been observed in *D. pyri*, *D. arabis*, *D. alopecuri* and *R. heterobia*.

7. Discussion.

It has been shown that in certain genera and species (in these genera) the number of antennal segments is perfectly constant. Such is the case in *Contarinia tritici* Kirby, *C. tragopogonis*, *C. merceri*, *Stenodiplosis geniculati*, *Sitodiplosis mosellana*, *Lestodiplosis achilleæ*, *Thomasiniana theobaldi*, and *T oculiperda*. It has been observed that individuals of these species show a variation in size, as would be expected in any animal population. It may be presumed that the food factor is implicated, the under-nourished individuals being of small size, while those better fed are of large size.

On the other hand, in species belonging to certain other genera the number of antennal segments is very variable within fixed limits. A species may have a range of from 2+15 to 2+11, with most individuals possessing 2+13 segments. This range of variation appears to be almost constant, but under some conditions most of the individuals may have 2+14 or 2+12 segments. In other words, the numbers within the antennal formula may vary, but the range does not. *Rhabdophaga heterobia*, *R. terminalis*, *Dasyneura alopecuri*, *D. pyri*, *D. arabis*, and *Colomyia hordei* are species that belong to this class.

It has been shown that the number of antennal segments is probably not inherited. Very strong circumstantial evidence has been given to indicate that there is a marked correlation between the number of antennal segments and the size of the midge.

Evidence has been given that the antennal formula of a species varies in different years, from different localities, on different host-plants, in different types of gall, in captivity, and, perhaps, under varying conditions of heat, cold, and light.

Since this variation in antennal formula is probably not inherited, and seems correlated in size, it can be suggested that the food factor is ultimately responsible for the variation in number of antennal segments. Now, arguing from this hypothesis, we can examine the evidence presented as to variation under different conditions. Firstly, we have seen that variation occurs from one year to another. It is reasonable to suppose that the condition of the host-plant (*i. e.*, food of the midge) is dependent on weather conditions, and varies in quality from year to year. Secondly, the variation due to locality: the host-plant may be of better food value in one place than in another. (Referring back to the cases given, it was shown that the formulæ for *D. alopecuri* varied in different localities in which the samples were picked. There is no evidence to show at what point in the larval stage of the midge these samples were picked. In some cases the larvæ might have been fully fed, in others a week from the fully-fed state, in another a fortnight from this state, and so on. This is a further suggestion that food plays an important part in the variation found in samples from different localities.) Thirdly, there is the variation in different types of gall. A fewer number of larvæ may be present in the "bud" galls of *R. heterobia* (observations support this) than in the "button" galls. If this is so, there might be more food for each, and so better developed adult midges might result. Fourthly, the variation on different host-plants might easily be due to the food factor. Similarly, midges bred in captivity for a number of generations are smaller and have fewer antennal segments than those reared from larvæ collected when fully fed under natural conditions. Fifthly, the suspected variation observed under conditions of extra heat or cold after the larvæ were fully fed might be explained by supposing that abnormal conditions would tend to have a lethal effect on the under-nourished larvæ, and only the better-fed ones would survive to the adult state.

It will be seen that all this evidence is strongly in favour of the food affecting the size of the midge and, in addition, the number of antennal segments in the species and genera where variation in number of antennal segments does occur.

If this be so it is remarkable, and of extraordinary interest, that in some genera food affects the size of adults only, and in others the size of adults and the number of antennal segments.

8. Conclusions.

1. In the Cecidomyidæ the number of antennal segments in certain species and genera is constant: in others it is variable.
2. The number of antennal segments is sometimes greater on one antenna than on the other. Imperfect differentiation of the segments is of usual occurrence.
3. It is possible to write a formula for the antennæ of variable species. In certain genera the males seem to have a greater number than the females, and in other genera *vice versa.*
4. In some species and genera food affects the size of the adult midges only: in others it affects the size of adult midges and, in addition, the number of antennal segments.

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EXPLANATION OF THE PLATE.

Plate I.

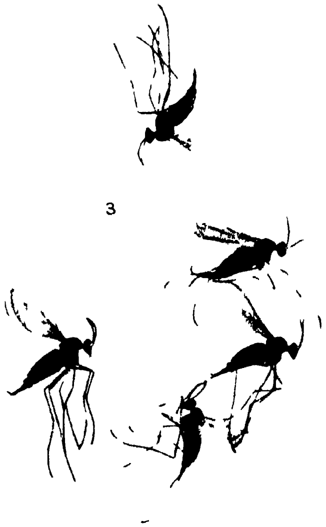
Groups of *R. terminalis* (♀ ♀) possessing different numbers of antennal segments to show correlation with size.

Fig. 1. Five individuals having 2 + 11 segments.
2. Others with 2 + 12.
3. Others with 2 + 13.
4. Others with 2 + 14.
5. Others with 2 + 15 antennal segments.

Magnification × 7

1

2



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A n d n

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THE LOSS OF TOXICITY OF PYRETHRUM DUSTS ON EXPOSURE TO AIR AND LIGHT.

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INTRODUCTION.

IT has been known for a long time that pyrethrum flowers (*Chrysanthemum cinerariaefolium*) lose their potency against insects under certain conditions of exposure. The rate at which this takes place has been found to vary; the unground flowers were shown by Abbott (1) to retain their toxicity 150 weeks when exposed in an open dish, whereas ground heads under the same conditions were practically worthless after this time. It was for a long time considered that the volatility of the active principles was the main cause of such loss, but the researches of Staudinger and Ruzicka (5), which elucidated the properties of the pyrethrins, definitely ruled this explanation out of court. The fact that the pyrethrins are complicated esters suggests other changes, such as hydrolysis, as a possible source of loss; on the other hand, these compounds are reducing substances and an alternative explanation might well be afforded by their oxidation. Other feasible explanations are intramolecular change or polymerisation, for it is known that small changes in constitution render the pyrethrins inactive against insects.

In a previous investigation Tattersfield and Hobson (7) examined the stability of pyrethrum extracts in solution or suspension in various solvents, and found them under these conditions to be comparatively stable both at ordinary temperatures and at 28–30° C. in closed vessels in the presence of various emulsifiers even when slightly alkaline. The active principles proved under these circumstances more stable than had been considered probable. It was found also that pyrethrum flowers, whether in the form of whole heads or as powder, retained their toxicity in closed vessels in the dark for considerable periods, even at 28° C., but that if spread in a thin layer as a finely ground powder and exposed to the air their insecticidal value was finally destroyed.

At this time we had also exposed to air a pyrethrum dust, in which the active principles were absorbed upon talc, and it was ascertained that the loss of activity in a preparation of this kind proceeded with great rapidity. The speed with which toxicity was lost called for investigation and at the same time facilitated experimental enquiry. It appeared that some such factor as polymerisation, intramolecular change or oxidation of the pyrethrins must play a part in the process. It was decided therefore to study the loss of activity in these dusts more thoroughly under a variety of different conditions. Further, if oxidation plays an active part in the reaction, it was possible that anti-oxidants might inhibit it partially or altogether, and the investigation was therefore extended to include a preliminary study of the effect upon the loss of toxicity, on exposure to air and light, of incorporating certain of these compounds with the dusts.

EXPERIMENTAL.

The experimental side of this investigation had three main aspects:

(1) The preparation of the dusts[1], etc., for exposure.

(2) The exposure of pyrethrum preparations to air, oxygen, carbon dioxide, nitrogen and *in vacuo*, both in the sunlight and in the dark.

(3) The testing of the samples to ascertain their insecticidal effect.

The preparation of the pyrethrum dusts and powders.

The experiments were carried out on extracts of pyrethrum taken up by absorbent earths, such as talc and kieselguhr, and upon the flowers ground to a relatively fine powder. The dusts were prepared by extracting pyrethrum flowers with petroleum ether and concentrating until the content in pyrethrins per 100 c.c. was equivalent to that of samples of flowers very rich in active principles. This extract was thoroughly incorporated with finely divided talc or white kieselguhr in such amounts as to give concentrations of the active principles equivalent to those of flowers of (*a*) a high quality (0·78 per cent. pyrethrin I[1]), (*b*) of moderately high quality (0·39 per cent. pyrethrin I). The incorporation was carried out either under artificial light or very rapidly in diffused daylight. The solvent was taken off in the shade or in the dark and the resultant powder stored in the dark in rubber-stoppered bottles or tubes. A small tube filled to the stopper with the dust was, whenever possible, set aside to act as an unexposed control for the subsequent series of experiments.

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[1] The term *dust* is used instead of *powder* to avoid confusion with ground pyrethrum flowers. At this stage they were prepared in such a form as to facilitate primarily a study of their loss of toxicity and not with the object of being most suitable for dusting.

When the effect of an anti-oxidant was to be tested it was added before or after the volatilisation of the petroleum ether, either in solution in some suitable solvent which could be volatilised, or it was incorporated through grinding.

Experiments with pyrethrum dusts.

A pyrethrum-talc dust (giving a volatile acid value calculated to 0·78 per cent. pyrethrin I[1]) was exposed in a glass-house in thin layers in Petri dishes (1) completely covered by an opaque box, *i.e.* in the dark, (2) to daylight, (3) to red light, (4) to blue light. The time of exposure was 3 days. After this period a known weight of the sample was extracted with an amount of absolute alcohol sufficient to give a 10 per cent. extract of the powder. The clear alcoholic solution was diluted with 0·5 per cent. saponin solution in water and tested biologically in the way previously outlined (8), using bean aphis as a test subject. The data are given in Table I.

The quality of the insects was not high enough to make it possible to draw very fine distinctions between the several tests, there being on the average 15 per cent. of insects which did not survive the control sprays, but the data of Table I definitely indicates that the incidence of light is requisite for the dusts to lose any material proportion of their activity. The data are not sufficiently good to give any clear and final proof as to whether any particular proportion of the spectrum is more effective than any other. Two of the samples were exposed under red and blue glass respectively, but neither of the glasses precluded entirely the transmission of other portions of the spectrum, and during the course of the experiment the illumination was frequently very bright sunlight. There was, however, some indication that the blue light was rather more effective than red light in destroying activity. Another series of tests was carried out with a talc dust containing an extract of pyrethrum but of about half the concentration in terms of pyrethrins of the samples dealt with in Table I. Equal amounts were placed in a well-stoppered tube, which was stored in the dark, and in three U-tubes, one of which was rendered opaque by covering the outside with a black paint; the three U-tubes were then placed in a thermostat kept at 25° C. Through one tube carbon dioxide was passed, through the other two (including the darkened tube) oxygen was passed each day for 12 days. From time to time the tubes were well shaken, and on one occasion the powder was taken out and well stirred in a mortar. It was noted that the sample exposed to oxygen and sunlight lost its yellow colour more rapidly than the others. At the end of the experiment, the dusts were extracted with alcohol and tests were made at the dilutions used in the experiment set out in Table I. Both bean aphis and an aphis found upon wheat were used as test subjects. (The tests upon the two species

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[1] The concentration was estimated by the short acid method of Tattersfield and Hobson (6). The acid, volatile in steam, was determined and calculated to pyrethrin I. This value is frequently referred to in this paper as volatile acid value calculated to percentage content of pyrethrin I.

Table I. *Toxicity to* Aphis rumicis *of extracts of pyrethrum-talc dusts after exposure in thin layers to sunlight and air for* 3 *days.*

N = not affected, *S* = slightly affected, *M* = moribund, *D* = apparently dead.

2 days after spraying.

Nature of experiment	Concentrations sprayed. Volatile acid calculated to pyrethrin I gm. per 100 c.c.	*N* %	*S* %	*M* %	*D* %	*M* and *D* %
Not exposed to air or light	0·002	—	—	—	100	100
	0·001	—	—	30	70	100
	0·0005	—	—	40* + 40	20	60–100
Exposed to air, and white light	0·002	100	—	—	—	0
	0·001	90	—	—	10	10
	0·0005	80	—	—	20	20
Exposed to air in dark	0·002	—	—	—	100	100
	0·001	—	—	—	100	100
	0·0005	10	40	20	30	50
Exposed to air in red light	0·002	—	—	30	70	100
	0·001	30	30	30	10	40
	0·0005	80	10	—	10	10
Exposed to air in blue light	0·002	10	30	50	10	60
	0·001	40	—	50	10	60
	0·0005	80	10	—	10	10
Controls with 0·5 % saponin and with mixtures of saponin and alcohol	—	85	—	10	5	15

* These insects were badly affected but not moribund.

of aphis were confirmatory of each other.) It is sufficient, however, to record that the only sample that could be regarded as having lost any of its activity was the one exposed to both light and oxygen; as, however, the light was diffused the loss was not great.

Tests carried out in closed vessels.

In view of these results a more elaborate series of experiments was made. A dust containing an extract of pyrethrum was prepared by thoroughly incorporating a highly concentrated extract of pyrethrum flowers in petroleum ether with a sample of kieselguhr in such quantity as to give a volatile acid value equivalent to about 0·4 per cent. pyrethrin I. The mixture was made rapidly, the petroleum ether taken off *in vacuo* in the dark, and finally stored in a well-stoppered darkened bottle in the dark. The powder was a friable yellow dust. 10 gm. of powder were weighed out as required into a series of 100 c.c. round-bottomed flasks. The following experiments were made:

1 (*a*). *Control in dark not exposed.* 10 gm. in rubber-stoppered tube kept in the dark during the course of the experiment.

1 (*b*) *Control exposed to air in dark.* 10 gm. in 100 c.c. round flask, kept in the shade (to act as control for the effect of handling in the subsequent experiments).

2 (*a*) *Exposure to wet oxygen.* 10 gm. in 100 c.c. round flask which was evacuated and *wet* oxygen admitted in the shade—flask sealed[1].

2 (*b*). *Exposure to dry oxygen.* 10 gm. in 100 c.c. round flask connected by means of a second small flask, filled with glass-wool, to a third containing phosphorus pentoxide, the whole system being evacuated to a few mm. pressure, oxygen passed over the phosphorus pentoxide into the system, the flasks sealed and the dust allowed to stand in the dark in the presence of the phosphorus pentoxide for 3 days with occasional turning over to expose a fresh surface. The sample was afterwards exposed to daylight in the presence of the drying agent.

3 (*a*). *Exposure to carbon dioxide.* 10 gm. in 100 c.c. round-bottomed flasks, evacuated once, carbon dioxide passed in—flask sealed.

3 (*b*). *Exposure to carbon dioxide.* 10 gm. in 100 c.c. flask evacuated—carbon dioxide passed in—re-evacuated and carbon dioxide again passed in—flask sealed.

4 (*a*). *Exposure* in vacuo. 10 gm. in 100 c.c. round-bottomed flask evacuated (pressure 2·8 mm.)—flask sealed.

4 (*b*). *Exposure* in vacuo *after carbon dioxide.* 10 gm. in 100 c.c. round-

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[1] The flasks were sealed by fusing inlet and outlet tubes in a blowpipe flame.

bottomed flask—evacuated—carbon dioxide passed in—re-evacuated (pressure 5·4 mm.)—flask sealed.

5. *Exposure in nitrogen.* 10 gm. in 100 c.c. round-bottomed flasks—nitrogen passed in for 10 min., evacuated and nitrogen re-passed for 10 min.—flask sealed.

All the flasks were stored in a cool dark room for 3 days, to allow the atmosphere and dust in experiment 2 (*b*) to stand for some time with the phosphorus pentoxide before exposure. Flasks 2 (*a*)–5 were afterwards laid on their sides in a tray in the glass-house and exposed to sunlight. The flasks were occasionally turned and gently shaken to ensure that a fresh surface was exposed. The exposure lasted for 7 days. The daily amounts of sunlight were rather variable, bright sunlight alternating with dull or variable overcast weather.

The Rothamsted records give the following figures for the amount of illumination.

	Bright sunlight hours	Callendar radiation recorder joules/cm.^{2}
August 18	11·2	1961
„ 19	2 7	832
„ 20	5 1	974
„ 21	2·8	888
„ 22	5 1	1181
„ 23	2 3	722
„ 24	0·8	913
„ 25	6·1	1187

At the end of 7 days the flasks were unsealed, a known weight of each dust was extracted with absolute alcohol to give a solution equivalent to 10 per cent. of the powder, dilutions were made and spray trials carried out upon bean aphis. It was noted that the alcoholic extracts had nearly the same depth of colour except in the case of samples exposed to oxygen and light, in which it was largely discharged.

The data given in Table II clearly indicate the important part played by oxygen and light in the loss of toxicity of these pyrethrum dusts. Little or no loss was observed in the samples exposed in the dark or *in vacuo*, or in carbon dioxide or nitrogen; such slight losses as were noted were hardly outside experimental error, and may well have been due to the presence of traces of oxygen in the inert gases or in the partial vacuum.

An interesting and unexpected feature of the data is the difference shown between 2 (*a*) and 2 (*b*). Although the results are not widely different, wet oxygen has proved somewhat less effective than dry oxygen. At this stage no explanation can be suggested for this result, but, perhaps, it should be placed on record that the pyrethrum-kieselguhr dust, during the exposure to wet oxygen, tended to adhere in a thin layer to the sides of the flasks on shaking and thus some diffusion of light may have taken place.

Table II. *Flask experiments. The effect of exposure of pyrethrum-kieselguhr dusts.*

Period of exposure, 7 days.

N = not affected, *S* = slightly affected, *M* = moribund, *D* = apparently dead.

2 days after spraying.

Nature of experiment	Concentrations sprayed. Volatile acid calculated to pyrethrin I gm. per 100 c.c.	*N* %	*S* %	*M* %	*D* %	*M* and *D* %
1 (*a*) Not exposed	0·002	—	—	—	100	100
	0·001	—	—	—	100	100
	0·0005	—	—	60	40	100
1 (*b*) Exposed to air in dark	0·002	—	—	—	100	100
	0·001	—	—	20	80	100
	0·0005	—	30	60	10	70
2 (*a*) Exposed to wet oxygen	0·002	—	40	50	10	60
	0·001	40	20	10	30	40
	0·0005	100	—	—	—	0
2 (*b*) Exposed to dry oxygen	0·002	40	10	50	—	50
	0·001	100	—	—	—	0
	0·0005	90	—	—	10	10
3 (*a*) Exposed to carbon dioxide	0·002	—	—	—	100	100
	0·001	—	—	30	70	100
	0·0005	—	20	70	10	80
3 (*b*) Exposed to carbon dioxide	0·002	—	—	—	100	100
	0·001	—	—	—	100	100
	0·0005	—	—	60	40	100
4 (*a*) Exposed *in vacuo*	0·002	—	—	—	100	100
	0·001	—	—	—	100	100
	0·0005	10	10	30	50	80
4 (*b*) Exposed *in vacuo*	0·002	—	—	10	90	100
	0·001	—	—	20	80	100
	0·0005	—	30	40	30	70
5 Exposed in nitrogen	0·002	—	—	—	100	100
	0·001	—	—	20	80	100
	0·0005	10	—	70	20	90
Controls—0·5 % saponin solution	—	100	—	—	—	0
Saponin and alcohol to correspond with highest concentration used	—	100	—	—	—	0
Saponin and alcohol to correspond with next highest concentration used	—	100	—	—	—	0

In addition, the ferricyanide reducing values were determined for these powders in the following way: 5 gm. of each powder was extracted with light petroleum ether, the ether taken off partly over an electric lamp in an atmosphere of CO_2 and finally *in vacuo*. The residue was treated with successive amounts of warm purified methyl alcohol, and after cooling and filtration the filtrate made up to 25 c.c. The methyl alcohol from 10 c.c. was evaporated off *in vacuo* and the residue extracted with 20 c.c. of aldehyde-free ethyl alcohol in successive amounts (4 c.c.) and made up to 20 c.c. with ethyl alcohol; after precipitation of proteins by zinc hydroxide the bulk was made up to 25 c.c., 10 c.c. taken and warmed in a Folin tube to 78° C. for 45 min. with 5 c.c. of an $N/10$ alkaline ferricyanide solution. After washing out with water and the addition of ferrocyanide precipitant and acetic acid, the solution was titrated with $N/50$ thiosulphate, using starch as indicator. The oxygen absorbed, taking the *unexposed* sample as unity, was as follows:

Unexposed		1 (*a*)	1·0
Exposed	in dark	1 (*b*)	0·83
,,	in wet oxygen	2 (*a*)	0·83
,,	in dry oxygen	2 (*b*)	0·57
,,	in carbon dioxide	3 (*a*)	0·86
,,	in carbon dioxide	3 (*b*)	0·89
,,	*in vacuo*	4 (*a*)	0·96
,,	*in vacuo*	4 (*b*)	0·84
,,	in nitrogen	5	0·89

Except for sample 2 (*b*), there is nothing in these figures, taken as a whole, from which any general deduction can be drawn. Unfortunately, a good deal of exposure was involved in the process of extraction, etc., which may have affected the results. What is noteworthy is the high oxygen absorption value of the sample 2 (*a*). Considering these figures in relation to the results of the biological tests, it is seen that this sample proved somewhat more toxic than sample 2 (*b*), but the toxicity is not commensurate with ferricyanide reduction, and it raises the question whether in this case hydrolysis of the esters has not taken place during exposure to sunlight in the presence of water vapour. Again, although the reduction value of 2 (*b*) (exposure to dry oxygen) shows so material a change as to demonstrate the important part played by oxygen absorption in the loss of toxicity, the result is nevertheless higher than would have been expected from the toxicity trials and would indicate that this method of analysis does not show completely the loss of insecticidal value due to exposure. Another experiment confirmed these conclusions; a sample of dust of double strength in active principles gave an oxidation absorption value of 2 (on the above basis) before exposure, but after exposure and the loss of a great portion of its activity, a value of 1·3.

This series of experiments makes abundantly clear the important part played by both light and oxygen in the loss of activity of pyrethrum dusts on exposure, but leaves undetermined the extent to which other factors, such as hydrolysis or polymerisation, may be involved under certain conditions.

Experiments with anti-oxidants.

Moureu and Dufraisse (3) in a long series of experiments have shown that certain chemicals act as protectors against oxidation; thus, to take one example, hydroquinone will greatly inhibit the oxidation of benzaldehyde. It seemed, therefore, to be worth while to ascertain whether the admixture of anti-oxidants with pyrethrum dusts and powders would protect them from loss of toxicity on exposure to light and air. Several series of experiments were carried out in which anti-oxidants were incorporated with (*a*) pyrethrum-talc dust, (*b*) pyrethrum-kieselguhr dust, (*c*) finely powdered pyrethrum flowers.

Series (*a*). A known volume of a concentrated petroleum ether extract of pyrethrum (50 c.c.) was absorbed on a weighed amount of finely ground talc (50 gm.) in diffused daylight and well mixed, the petroleum ether allowed to evaporate in the dark and the resulting dust again well mixed (A). This was equivalent to a high-grade pyrethrum (the volatile acid figure calculated to pyrethrin I being 0·79 per cent.). To similarly prepared mixtures were added 2·5 gm. and 0·5 gm. of hydroquinone (B 1 and B 2) in solution in ether, the ether being allowed to evaporate in the dark. Another dust was prepared in which 0·5 gm. of colloidal sulphur was incorporated (*C*). As the colloidal sulphur was suspended in water the mixture made from it was dried *in vacuo* over calcium chloride. Each sample was divided into two parts, one of each being stored in tubes in the dark and the other exposed in a thin layer in large Petri dishes in a glass-house for 13 days, the samples being stirred each day. Known weights of the exposed and unexposed samples were extracted with absolute alcohol and diluted with 0·5 per cent. saponin solution and sprayed upon *Aphis rumicis*. During the course of the experiment it was noted that, in the case of the untreated samples and the one containing sulphur, the characteristic yellow colour of the dust was rapidly destroyed. The bleaching effect did not extend much below the surface, but at the end of the experiment the samples A and C were almost white throughout, whereas the mixtures with hydroquinone still retained a distinct yellow shade tinged with grey. The insecticidal results of these experiments are given in Table III.

Table III. *The effect of exposure on the toxicity of pyrethrum-talc dusts with and without anti-oxidants.*

Time of exposure, 13 days in Petri dishes.
Insects used—wild *Aphis rumicis.*

N = not affected, *S* = slightly affected, *M* = moribund, *D* = apparently dead.
Results 3 days after spraying.

Experiment	Concentrations sprayed. Volatile acid calculated to pyrethrin I gm. per 100 c.c.	*N* %	*S* %	*M* %	*D* %	*M* and *D* %
A. No treatment. Dust not exposed to air or light	0·002	—	—	20	80	100
	0·0015	—	—	40	60	100
	0·001	20	20	50	10	60
	0·00075	10	30	50	10	60?
A. No treatment. After exposure to air and light	0·002	100	—	—	—	0
	0·0015	90	—	—	10	10
	0·001	100	—	—	—	0
	0·00075	100	—	—	—	0
B 1. Mixture contains 5 % hydroquinone. No exposure	0·002	—	—	10	90	100
	0·0015	—	—	—	100	100
	0·001	—	—	20	80	100
	0·00075	—	20	80	—	80
B 1. Mixture contains 5 % hydroquinone. After exposure to air and light	0·002	—	—	80	20	100
	0·0015	—	10	70	20	90
	0·001	10	30	60	—	60
	0·00075	50	20	10	10	20
B 2. Mixture contains 1 % hydroquinone. No exposure	0·002	—	—	60	40	100
	0·0015	—	10	60	30	90
	0·001	—	30	50	20	70
	0·00075	10	50	40	—	40
B 2. Mixture contains 1 % hydroquinone After exposure to air and light	0·002	—	—	40	60	100
	0·0015	30	—	60	10	70
	0·001	60	10	10	20	30
	0·00075	70	30	—	—	0
C. Mixture contains 1 % colloidal sulphur. No exposure	0·002	10	—	30	60	90
	0·0015	—	—	60	40	100
	0·001	20	10	50	20	70
	0·00075	20	50	30	—	30
C. Mixture contains 1 % colloidal sulphur. After exposure to air and light	0·002	90	—	—	10	10
	0·0015	90	—	10	—	10
	0·001	90	—	—	10	10
	0·00075	100	—	—	—	0
Control with saponin 0·5 %	—	100	—	—	—	0
Control with saponin + 10 % alcohol	—	90	—	—	10	10

The insects used in this experiment were a wild colony. Owing to the risk of parasitism, it is our usual practice to employ insects reared in captivity, but owing to our stock reproducing at an extremely slow rate during the cold and rather sunless spring and summer of 1931 and having also a lower resistance than usual, we took advantage of a supply of wild insects for these trials, where large differences in the results were expected. There was a very small amount of parasitism, but, as the parasitised insects were readily recognised and eliminated, not sufficient materially to affect the results. On the whole, the insects were of good quality; they were of higher resistance than the ordinary supply for 1931 and therefore the figures given in this table should not be compared with the data in others.

The figures definitely demonstrate (1) that in 13 days the pyrethrum-talc dust, unmixed with any anti-oxidant, has lost on exposure a very large proportion, if not all, its activity; (2) that an admixture of 5 per cent. of hydroquinone has preserved almost the whole of the activity for that period; (3) that an admixture of 1 per cent. of hydroquinone while not so effective as the 5 per cent. mixture has secured a good measure of protection; (4) that colloidal sulphur has given no protection; (5) that the dust containing 5 per cent. hydroquinone, but not exposed to air and light, has given an extract having a somewhat higher measure of toxicity than the sample with no such admixture, an indication that probably the active principles have been protected even after the application of the spray and that the addition of anti-oxidants may increase the effectiveness of alcoholic extracts of pyrethrum.

A second series of experiments with hydroquinone, tannic acid and paraldehyde were carried out. A dust, containing talc and pyrethrum extract, was prepared by absorbing a concentrated petroleum ether extract of pyrethrum flowers upon finely powdered talc, the petroleum ether being subsequently taken off *in vacuo* in the dark and the dust afterwards well mixed in a mortar. To weighed amounts were added (*a*) 2·5 per cent. hydroquinone in a strong solution in ether, (*b*) 2·5 per cent. tannic acid in alcohol, (*c*) 2·5 per cent. paraldehyde; in addition, known weights of pure finely ground talc (without pyrethrum) were mixed in the same way with the same percentage amounts of these chemicals. Each of the pyrethrum-talc preparations and the talc powder containing the chemicals were divided into two portions, one of which was stored in well-stoppered tubes and the other exposed to air and light in a thin layer in a Petri dish. The samples were exposed with occasional stirring for 3 days in bright sunlight, after which known weights were extracted with absolute alcohol and their insecticidal value tested in the usual way. Paraldehyde afforded no protection that could be detected, and the data in this case were not included in Table IV.

Table IV. *The protective effect of hydroquinone and tannic acid on pyrethrum-talc dust against exposure to light and air for* 3 *days.*

N = not affected, *S* = slightly affected, *M* = moribund, *D* = apparently dead. Results 2 days after spraying.

Experiment	Concentrations sprayed. Volatile acid calculated to pyrethrin I gm. per 100 c.c.	*N* %	*S* %	*M* %	*D* %	*M* and *D* %
Pyrethrum-talc dust. No treatment. Not exposed	0·002	—	—	—	100	100
	0·001	—	—	30	70	100
	0·0005	—	—	40* + 40	20	60–100
Pyrethrum-talc dust. No treatment. Exposed to light and air	0·002	100	—	—	—	0
	0·001	90	—	—	10?	10?
	0·0005	80	—	—	20	20
Pyrethrum-talc dust. Treated with 2·5 % hydroquinone. Not exposed	0·002	—	—	—	100	100
	0·001	—	—	—	100	100
	0·0005	10	10	50	30	80
Pyrethrum-talc dust. Treated with 2·5 % hydroquinone. Exposed to light and air	0·002	—	—	—	100	100
	0·001	10	—	20	70	90
	0·0005	20	10	60	10	70
Talc dust and 2·5 % hydroquinone. Not exposed	—	100	—	—	—	0
Talc dust and 2·5 % hydroquinone. Exposed	—	80	—	10	10	20
Pyrethrum-talc dust. Treated with 2·5 % tannic acid. Not exposed	0·002	—	—	—	100	100
	0·001	—	—	—	100	100
	0·0005	—	—	30	70	100
Pyrethrum-talc dust. Treated with 2·5 % tannic acid. Exposed	0·002	—	—	30	70	100
	0·001	10	10	50	30	80
	0·0005	70	—	20	10	30
Talc dust and 2·5 % tannic acid. Not exposed	—	90	—	—	10	10
Talc dust and 2·5 % tannic acid. Exposed	—	100	—	—	—	0
Saponin 0·5 %. Average	—	90	—	—	10	10
Saponin and alcohol. Average	—	80	—	20	—	20

* These insects were seriously affected but not moribund.

The insects used for the tests expressed in Table IV were not very highly resistant, and there were more deaths in the control sprays than usual. Only large differences in the results were therefore regarded as valid for purposes of drawing conclusions. It will be seen that the untreated pyrethrum-talc dust has almost completely lost its toxicity on exposure to air and bright sunlight, whereas the sample treated with hydroquinone has only been slightly affected, and although the pyrethrum-talc dust with which tannic acid was incorporated does show some slight loss, the protective power of this compound is obviously considerable. The chemicals by themselves either before or after exposure do not give results markedly different from the controls and may be regarded as having little or no toxicity. On the last day a note of the depth of colour of each of the exposed pyrethrum-talc dusts was recorded, the order being as follows:

Hydroquinone > tannic acid > paraldehyde = no treatment.

Although no direct connection has been found between pyrethrin content and the colour of the samples, it is obvious that oxidation plays some part in the colour change and that loss of colour can be partially inhibited by the use of anti-oxidants.

In view of the above results, a larger series of experiments was set up to test the relative oxidation-inhibiting power of certain anti-oxidants, particularly the hydroxy-benzenes. A kieselguhr-pyrethrum dust was prepared of half the strength (in terms of active principles) of those used in obtaining the data set out in Tables III and IV. It was prepared in a dark room by absorbing upon white kieselguhr a strong petroleum ether extract of pyrethrum, the petroleum ether was taken off in the dark *in vacuo*, the dust was finally well mixed in a mortar and stored in the dark. To 10 gm. of powder there were added in the dark 0·25 gm. of the following reagents (where a solvent was used it was in each case taken off *in vacuo* in the dark).

Monohydroxy benzenes:

1. Phenol in strong solution in ether.

Dihydroxy benzenes:

2 (*a*). Pyrocatechol in strong solution in ether.
2 (*b*). Resorcinol in strong solution in ether.
2 (*c*). Hydroquinone in strong solution in ether.

Trihydroxy benzenes:

3 (*a*). Pyrogallol in solution in ether.
3 (*b*). Phloroglucinol in solution in ether.
4 (*a*). Tannic acid in solution in alcohol.
4 (*b*). Tannic acid as powder well ground in.
5 (*a*). Myrabolan powder 0·5 gm., ground in.
5 (*b*). Cutch powder 0·5 gm., ground in.

In addition, mixtures of kieselguhr *alone* with the same amounts of chemicals were made in the same way.

It should be noted that some samples of ether which have been long exposed to light and air may be unsuitable as solvents for polyhydroxy phenols, as the possible presence of peroxides affects the anti-oxidants.

The samples were exposed to sunlight and air in a thin layer in Petri dishes with occasional stirring to expose a fresh surface. In order that the untreated samples should retain some of their activity after exposure, the samples were allowed to remain out in the glass-house for a period of 2 days only. They were then extracted by alcohol and tested in the usual way. The data for the polyhydroxy benzenes Nos. 1–3 are set out in Table V A, *Aphis rumicis* being used in these experiments, and for the tannic acid, etc. (Nos 4, 5), in Table V B, the test subject being an aphis found on wheat (*Aphis pardi?*).

The data are presented in two tables, as owing to shortage of *Aphis rumicis* suitable for spraying, the whole of the material could not be tried out with that insect. The *Aphis rumicis* used came from a wild colony, and there was a small amount of parasitism (about 1 per cent.) which had some slight effect upon the results, but as large differences only were sought, this adds no appreciable difficulty to the interpretation of the results. Two days' exposure has not been sufficient to deprive the untreated kieselguhr-pyrethrum dust of the whole of its activity, nevertheless it has disappeared to a considerable extent. A comparison of the figures show that the three dihydroxy benzenes, pyrocatechol, resorcinol and hydroquinone and the trihydroxy benzene pyrogallol afford a very high degree of protection, whereas phenol and phloroglucinol give little or none. The result given by phenol was not unexpected, for although it is stated to have anti-oxidising properties, these are not considerable; the poor protection capacity of phloroglucinol is not easy to explain; it is, however, a desmotropic substance of the keto-enol type, and it may be tentatively suggested that its comparatively poor protective power may be in some way associated with this type of tautomerism.

The data in Table V B, demonstrate the protective power of tannic acid, whether incorporated with the dust in solution or as a solid powder with subsequent attrition. The tannic acid molecule contains polyhydroxy benzene nuclei, and it would appear certain that it derives its anti-oxidising power from this fact. Both myrabolan and cutch powders offer some measure of protection, the former is a source of tannin and the latter contains polyhydroxy phenol groupings to which its protective properties are unquestionably due.

That the effect of these derivatives is due to their anti-oxidant

Table V A. *Protective power of various anti-oxidants upon kieselguhr-pyrethrum dusts.*

Aphis rumicis used as test subject.

N = not affected, S = slightly affected, M = moribund, D = apparently dead.

Results 2 days after spraying.

Experiment	Concentrations sprayed. Volatile acid calculated to pyrethrin I gm. per 100 c.c.	N %	S %	M %	D %	M and D %
Kieselguhr-pyrethrum dust. No treatment. Not exposed	0·002	—	—	—	100	100
	0·001	—	—	20	80	100
	0·0005	—	—	40	60	100
Kieselguhr-pyrethrum dust. No treatment. Exposed 2 days	0·002	—	—	50	50	100
	0·001	60	20	20	—	20
	0·0005	80	10	—	10	10
1. Kieselguhr-pyrethrum dust. Treatment with phenol 2·5 % in ether. Exposed 2 days	0·002	10	10	30	50	80
	0·001	50	20	20	10	30
	0·0005	60	10	10	20	30
Kieselguhr + 2·5 % phenol. Exposed 2 days	To correspond with 1 conc.					
	0·002	90	—	—	10	10
2 (*a*) Kieselguhr-pyrethrum dust. Treatment with pyrocatechol 2·5 %. Exposed 2 days	0·002	—	—	—	100	100
	0·001	—	—	—	100	100
	0·0005	—	—	30	70	100
Kieselguhr + 2·5 % pyrocatechol. Exposed 2 days	To correspond with 2 *a* conc.					
	0·002	90	—	—	10	10
2 (*b*) Kieselguhr-pyrethrum dust. Treatment with resorcinol 2·5 % in ether. Exposed 2 days	0·002	—	—	—	100	100
	0·001	—	—	20	80	100
	0·0005	10	—	20	70	90
Kieselguhr + 2·5 % resorcinol. Exposed 2 days	To correspond with 2 *b* conc.					
	0·002	80	—	—	20	20
2 (*c*) Kieselguhr-pyrethrum dust. Treatment with hydroquinone 2·5 % in ether. Exposed 2 days	0·002	—	—	—	100	100
	0·001	—	—	—	100	100
	0·0005	—	—	20	80	100
Kieselguhr + 2·5 % hydroquinone. Exposed 2 days	To correspond with 2 *c* conc.					
	0·002	90	—	10	—	10
3 (*a*) Kieselguhr-pyrethrum dust. Treatment with pyrogallol 2·5 % in ether. Exposed 2 days	0·002	—	—	—	100	100
	0·001	—	—	—	100	100
	0·0005	—	—	20	80	100
Kieselguhr + 2·5 % pyrogallol. Exposed 2 days	To correspond with 3 *a* conc.					
	0·002	90	—	—	10	10
3 (*b*) Kieselguhr-pyrethrum dust. Treatment with phloroglucinol 2·5 % in ether. Exposed 2 days	0·002	30	20	30	20	50
	0·001	70	10	10	10	20
	0·0005	40	30	—	30	30
Kieselguhr + 2·5 % phloroglucinol. Exposed 2 days	To correspond with 3 *b* conc.					
	0·002	80	—	—	20	20
Saponin solution 0·5 %	—	90	—	—	10	10

Table VB. *Protective power of various anti-oxidants upon kieselguhr-pyrethrum dusts.*

An aphis upon wheat used as test-subject.

N = not affected, S = slightly affected, M = moribund, D = apparently dead.

Results 1 day after spraying.

Experiment	Concentrations sprayed. Volatile acid calculated to pyrethrin I gm. per 100 c.c.	N %	S %	M %	D %	M and D %
Kieselguhr-pyrethrum dust. No treatment. Not exposed	0·002	—	—	50	50	100
	0·001	—	10	70	20	90
	0·0005	—	20	30	50	80
Kieselguhr-pyrethrum dust. No treatment. Exposed 2 days	0·002	10	20	40	30	70
	0·001	40	30	20	10	30
	0·0005	50	20	10	20	30
4 (*a*) Kieselguhr-pyrethrum dust. Treatment with 2·5 % tannic acid in alcohol. Exposed 2 days	—	—	—	80	20	100
	—	—	—	60	40	100
	—	10	30	30	30	60
4 (*b*) Kieselguhr-pyrethrum dust. Treatment with 2·5 % tannic acid ground in. Exposed 2 days	—	—	—	80	20	100
	—	—	—	90	10	100
	—	10	20	40	30	70
Kieselguhr + 2·5 % tannic acid. Exposed 2 days	To correspond with 4*b* conc. 0·002	90	10	—	—	0
5 (*a*) Kieselguhr-pyrethrum dust. Treatment with 5 % myrabolan powder. Exposed 2 days	0·002	10	—	60	30	90
	0·001	—	50	50	—	50
	0·0005	50	10	—	30	30
Kieselguhr + 5 % myrabolan powder. Exposed 2 days	To correspond with 5*a* conc. 0·002	90	10	—	—	0
5 (*b*) Kieselguhr-pyrethrum dust. Treatment with 5 % cutch powder. Exposed 2 days	0·002	—	10	90	—	90
	0·001	10	30	40	20	60
	0·0005	50	30	10	10	20
Kieselguhr + 5 % cutch powder. Exposed 2 days	To correspond with 5*b* conc. 0·002	100	—	—	—	0
Saponin solution 0·5 %	—	90	—	10	—	10

properties should be indicated by the higher ferricyanide reducing values after exposure, when compared with those of untreated samples. It was only possible to carry out one estimation for which talc dust treated with hydroquinone was chosen. The first four samples in Table IV (p. 407) were taken, extracted with petroleum ether and afterwards treated in the way described on p. 403. If the value for the untreated *unexposed* sample is taken as unity, the untreated *exposed* sample gave 0·66; the *unexposed* sample, treated with hydroquinone, gave 1·04; the *exposed* sample, treated with hydroquinone, gave 1·08.

The loss of toxicity of powdered pyrethrum flowers.

Some experiments were also carried out on powdered flower-heads, which were found to lose their toxicity at a slower rate than the artificially prepared dusts. One sample, exposed for 3 months in a Petri dish in the glass-house used for these experiments, had only lost part of its activity at the end of this time. It seemed advisable in view of this to attempt to ascertain whether an anti-oxidant was playing a part in slowing up the loss of activity, or whether the effect was due to larger particle size or cellular inclusion. For this purpose a finely ground sample of pyrethrum was extracted by petroleum ether and subsequently with absolute alcohol. From these extracts were prepared three talc dusts equivalent to the original flowers in:

(1) Content of petroleum ether extract.

(2) Content of petroleum ether extract and subsequent alcoholic extract.

(3) Content of subsequent alcoholic extract.

One half of the sample of treated talc dust and of the original flowers was set aside in tubes in the dark and the other spread in a thin layer in Petri dishes and exposed in the glass-house for 4 days. After this period the samples were extracted and tested in the way previously described.

The data presented in Table VI for bean aphis as a test subject were confirmed by the results of the separate series of tests in which wheat aphis were used. They show that the active principles in the powdered flowers lose their activity at a slower rate than when absorbed upon talc, and that the addition of the alcoholic extract of the flowers already exhausted by petroleum ether has a slight, but only a slight, stabilising effect. The figures are not sufficiently good to make very fine distinctions as there seems to have been some slight loss in the unexposed sample treated with the alcoholic extract; this may have arisen from the mechanical difficulty met with in absorbing the alcoholic extract by the talc, already treated with the petroleum ether extract. Thus from these experiments it cannot be claimed that pyrethrum flowers contain any very efficient stabiliser, and it would appear that the superior stability of the flowers over the dusts is mainly due to larger particle size or to the fact that they contain much of the active principles as cellular inclusions.

Table VI. *Comparison between powdered pyrethrum flowers and pyrethrum-talc dusts in their loss of activity on exposure.*

Test-subject, *Aphis rumicis.*

N = not affected, *S* = slightly affected, *M* — moribund, *D* = apparently dead.

Results 2 days after spraying.

Experiment	Concentrations sprayed. Volatile acid calculated to pyrethrin I gm. per 100 c.c.	*N* %	*S* %	*M* %	*D* %	*M* and *D* %
Powdered pyrethrum flowers. Not exposed	0·002	—	—	—	100	100
	0·001	—	10	30	60	90
	0·0005	—	10	80	10	90
Powdered pyrethrum flowers. Exposed 4 days	0·002	—	—	30	70	100
	0·001	—	—	40	60	100
	0·0005	60	10	10	20	30
Petroleum ether extract of above flowers absorbed on talc. Not exposed	0·002	—	—	—	100	100
	0·001	—	—	10	90	100
	0·0005	—	—	60	40	100
Petroleum ether extract of above flowers absorbed on talc. Exposed 4 days	0·002	80	10	—	10	10
	0·001	90	—	—	10	10
	0·0005	100	—	—	—	0
Petroleum ether extract of above flowers absorbed on talc + alcoholic extract of above flowers after petroleum ether extract. Not exposed	0·002	—	—	—	100	100
	0·001	—	—	40	60	100
	0·0005	60	—	20	20	40
Petroleum ether extract of above flowers absorbed on talc + alcoholic extract of above flowers after petroleum ether extract. Exposed 4 days	0·002	10	40	30	20	50
	0·001	70	10	—	20	20
	0·0005	90	—	—	10	10
Talc + alcoholic extract of pyrethrum flowers after petroleum ether extract. Not exposed	Concentration to correspond with 0·002	80		—	20	20
Talc + alcoholic extract of pyrethrum flowers after petroleum ether extract. Exposed 4 days	11	80	—	—	20	20
Saponin 0·5 %	—	90	—	—	10	10

Effect of anti-oxidants in stabilising the activity of ground pyrethrum flowers.

There is greater difficulty in measuring the effect of anti-oxidants upon the powdered flower-heads because loss of toxicity is slower than in the case of dusts. It was only found possible to carry out one series of preliminary experiments in which hydroquinone and tannic acid were ground into a sample of finely ground heads. The experiment was carried out as before and an exposure of 8 days was made. Although the data were not completely conclusive, some evidence that the active principles were at least partially stabilised was forthcoming. When sprayed at concentrations of 0·001 per cent. pyrethrin I, the following percentages of moribund and dead insects were obtained 2 days after spraying:

Untreated	—not exposed	100
Untreated	—exposed	0
Treated with hydroquinone—	„	30
„ pyrogallol —	„	40
„ tannic acid —	„	40–50?
Control saponin 0·5 per cent.		5
Control saponin and alcohol		0

CONCLUSIONS.

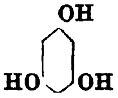
Our investigations have so far been of a preliminary nature, but it is hoped to extend the observations, for the loss of toxicity of pyrethrum powders has often borne an illusive aspect, and a more critical examination of the whole problem from the different angles touched on above seems worth while. So far we have not been able to detect any material loss in the absence of oxygen or in the dark, and the rough experiment of exposing dusts in two different coloured lights, while suggestive, was not conclusive. It is obvious that a study from a dynamical and a more strictly photochemical point of view is necessary, both as applied to dusts and solutions. The difficulties, however, are likely to be considerable, for in the simpler problem of the oxidation of benzaldehyde Kothari and Watson (2) state that the greatest difficulty was encountered in the inconsistency of their results obtained under apparently identical conditions, minute quantities of impurities in the benzaldehyde apparently affecting the results.

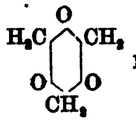
For loss of toxicity of pyrethrum dusts it is evident from our results that both light and air are required, as samples, exposed to inert gases or *in vacuo*, showed little loss in the times of exposure employed. Nevertheless, the possibility of loss of toxicity due to intramolecular change is not entirely ruled out, although our data would indicate that it plays a relatively unimportant part. The ferricyanide reduction figures showed too a lowered oxygen absorption value after exposure to air and light, nevertheless there is still a considerable proportion of reducing value left in the extracts after exposure, indicating that the ferricyanide method, suggested by Martin and Tattersfield (4), would not detect loss of toxicity in its entirety.

The rôle played by moisture in the loss of activity is still left obscure. In our tests in the presence of moist air the loss of toxicity was slightly less than when dry oxygen was used. It is true that the experiment is an isolated one, but the ferricyanide reduction value of the sample exposed to wet oxygen was markedly higher than the samples exposed to dry oxygen, and the possibility arises that, under normal exposure to the atmosphere, the process may have a considerable complexity, the course of the reaction depending upon the degree of humidity and the degree and nature of the illumination, although from our experiments oxidation would appear to play the predominant part.

Seeing that light activates the reaction, the absorption spectra of the samples is obviously of importance, and the effect of the degree of absorption may play an important part. White absorbent earths were used in our experiments and it was noted that the colour discharge was limited to the surface; an examination of the depth to which the effect penetrated when the extracts are absorbed upon differently coloured earths or upon surfaces with different light absorptions would prove of considerable interest.

In our experience, the finely ground pyrethrum flowers were less readily inactivated than artificially prepared dusts, and it would appear that the finer the powder the more readily inactivation takes place in sunlight and air. So far we have no clear evidence that anti-oxidants play an important part in the protection of the pyrethrins in the flowers, although it has been suggested that metabolic processes in living plant cells may well be regulated by their presence. Particle size and cellular inclusion would appear to play a more important protecting rôle. Further enquiry, however, into this aspect of the case is required.

That anti-oxidants do protect the activity of pyrethrum dusts is clear from the evidence presented here. Fine differences in the degree of protection were not sought, and all that can be said at this stage, as to the various substances tested, is that among the phenolic bodies the derivatives pyrocatechol, resorcinol, hydroquinone, pyrogallol give a very considerable measure of protection, whereas phenol and phloroglucinol do not. The behaviour of phloroglucinol was unexpected and the reason for failure awaits elucidation. It may well be due to either physical or structural causes. It was applied in ether in which solvent it is not readily soluble—it thus may have separated out in crystals of a relatively large size and so may have presented a small protecting surface. Phloroglucinol is, however, known to undergo a desmotropic change of the keto-enol order and it may be that, whereas the structure of the phenol type  affords protection, the isomeric quinone



might either be inactive or even accelerate to some extent the oxidation of the active principles.

Tannic acid had a powerful protective action, unquestionably due to the presence of polyhydroxy nuclei in its molecular make-up.

SUMMARY.

1. Pyrethrum powders and dusts, prepared by grinding or by the incorporation of extracts of pyrethrum flowers upon absorbent earths, such as talc and kieselguhr, lose their insecticidal activity on exposure to light and air. The loss is more rapid in the case of artificially prepared dusts than with ground flower-heads.

2. Both light and air play an important part in the process of inactivation, as samples of kieselguhr-pyrethrum and talc-pyrethrum dusts stored in closed vessels in the dark or exposed to air in the dark are relatively stable; also samples exposed to light in an atmosphere of carbon dioxide, nitrogen or *in vacuo* lose little of their toxicity under the same conditions of illumination; samples exposed in oxygen, however, rapidly lose their activity.

3. Both wet and dry oxygen were effective in destroying the activity of the dusts, but apparently at different rates, and there is some suggestion that the type of reaction may be different in the two cases.

4. The incorporation of anti-oxidants with talc-pyrethrum and kieselguhr-pyrethrum dusts retards loss of activity due to exposure to light and air.

5. Such compounds as pyrocatechol, resorcinol, hydroquinone, pyrogallol confer a large measure of protection against loss of toxicity. Phenol and phloroglucinol were not effective.

6. Tannic acid exerted a considerable measure of protection.

7. The protection was greater in the case of artificially prepared dusts than with ground pyrethrum flowers, although it seems also to be exerted to some extent in the latter case.

8. There is no conclusive evidence that anti-oxidants, naturally occurring in pyrethrum, play any great part in stabilising the pyrethrins against inactivation. The greater part of the protection would appear to be due to particle size or to cellular inclusion.

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THE INSECTICIDAL PROPERTIES OF *TEPHROSIA MACROPODA* HARV. AND OTHER TROPICAL PLANTS

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During the past few years a considerable number of plants received from different parts of the Empire and elsewhere have been examined for their toxicity to insects. Most of these plants belong to the order Leguminosae; they come chiefly from tropical countries and many of them are known to be employed by the natives of the districts where they occur as "fish poisons." A few plants belonging to other natural orders and some which are not fish poisons have been included.

The best known of these fish-poison plants is *Derris* or tuba-root, which is already widely used as an insecticide; others possessing high insecticidal powers and possibly of commercial importance are *Tephrosia vogellii* Hooker, *T. toxicaria* Pers. and species of *Lonchocarpus*[1] known as black and white Haiari. These have been the subjects of separate investigations, the results of which have already been published (1).

Among numerous other plants examined, the great majority proved to be without insecticidal properties or to possess them only in a slight degree, but a few were definitely toxic to insects, and may be worth further investigation. It is thought that the data accumulated should be put on record, although detailed studies of these plants have not at present been attempted.

In making preliminary trials, the plant material was ground finely, extracted with alcohol or water, and the extracts diluted with a 0·5 per cent. solution of non-toxic saponin. A high concentration (equivalent to 1–5 per cent. of the plant material) was tried in the first place. Laboratory spraying tests were then carried out with these extracts by means of the apparatus and method which have been already described (2). The insect used in most of the experiments was the Black Bean Aphis

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[1] Another species of *Lonchocarpus*, known as Cubé, has also been found to be very toxic to insects and has been studied in America.

(*Aphis rumicis* L.), feeding on Broad Bean plants, and bred as far as possible under standardised conditions.

The following is a list of plants[1] samples of which showed little or no toxicity to *Aphis rumicis* when tested under these conditions.

Species	Natural order	Derivation	Parts tested
**Albizzia stipulata* Boiv.	Leguminosae	India	Leaves and bark
**A. procera* Benth.	"	"	
**Acacia pruinescens* Kurz.	"	Burma	
**A. salicina* Lindl.	"	Australia	specs.)
A. falciformis D.C.	"	"	"
**A. pennata* Willd.	"	Burma	"
Caconcia coccinea	Combretaceae	British Guiana	Shell and kernel of fruit
*†*Cassia didymobotria* Fres.	Leguminosae	Kenya	Roots, stems, leaves, seed
**C. hirsuta* L.	"	Malaya	"
**Clibadium vargesii* (known as Nivrai)	Compositae	Antigua	Leaves, stems
†*Clitoria macrophylla* Wall.	Leguminosae	Siam	Roots
Derris scandens	"	British Guiana	Roots, stems, leaves
**D. trifoliata* (*uliginosa*)	"	India, Siam	Branches, roots, leaves
**Dolichos lupiniflorus*	"	Southern Rhodesia	Roots
Drepanocarpus lunatus	"	British Guiana	Leaves, stems, roots, fruit
**Euphorbia hiberna* L.	Euphorbiaceae	Ireland	Stems, leaves
**E. cotinoides* (known as Conaparu)	"	British Guiana	"
Haronga paniculata Lodd.	Guttiferae	Sierra Leone	Bark
Jacquinia ruscifolia	Theophrastaceae	British Guiana	Stems, leaves
**Lonchocarpus latifolius* Pers.	Leguminosae	Trinidad	Seeds, pods
*† "	"	—	"
†*Mammea americana* L.	Guttiferae	Trinidad	Roots, shoots, branches
†*Melia azadirachta* L.	Meliaceae	India	Leaves
**Milletia pachycarpa* Benth.	Leguminosae	Burma	Bark
*†*Neurolaena lobata* (known as Erb-à-picque)	Compositae	Antigua	Leaves, stems
**Ougeinia dalbergioides* Benth.	Leguminosae	India	Leaves, bark
Chrysanthemum (*Pyrethrum*) *frutescens*	Compositae	Uganda	"
**Phyllanthus conami* Sw. (known as Danconami or Daukanani)	Euphorbiaceae	British Guiana	Roots, stems, leaves
*†*Pithecolobium elliptica* Hassk.	Leguminosae	Malaya	Leaves, bark
Physalis angulata	Solanaceae	British Guiana	Whole of plant
Pulicaria dysenterica Gaertn.	Compositae	England	Leaves, flowers, stems, roots
Stemona collinsae Craib.	Stemonaceae	Siam	Tubers
*†*Tephrosia candida*	Leguminosae	Trinidad	Stems, roots
**T. purpurea* L.	"	India?	Roots, stems, leaves
**T. heckmanniana*	"	Southern Rhodesia	Stems and leaves together
*†*T. hookeriana*	"	"	Roots, stems, leaves, fruits
Uvaria latifolia Prain	Anonaceae	Siam	Roots
*"*Conami clibadium*" (Clibadium ? Surinamense)	Compositae	British Guiana	Roots, stems, leaves, flowers, fruit
*"*Hebitchioahabu*" (*Serjania* sp.)	Sapindaceae	"	Stems
*"*Moroballi*" (*Muraballi*) (*Cupania* sp.?)	Sapindaceae?	"	Wood, bark

The precise identity of the last three plants is doubtful. Plants known to be fish poisons are marked *. Plants showing some slight toxicity are marked †.

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[1] All plants from abroad were received in dry condition. It is possible that infusions of fresh leaves or stems might in some cases give different results.

The preliminary experiments with these plants having given negative, or almost negative results, no further time was spent on them. It is possible, however, that *Lonchocarpus latifolius* would justify further examination; the seeds and pods showed some slight toxicity and, in view of the powerful insecticidal properties possessed by certain other members of the genus, it would be of interest to test the leaves, stems and roots of this plant. The seeds of *Tephrosia hookeriana* also showed some toxicity.

Aqueous infusions of the fresh leaves of *Tephrosia heckmannia* were reported by Mr H. C. Arnold of Southern Rhodesia *in litt.* to have toxicity to bed bugs and to larvae of the maize stalk borer; but extracts of the dry material received were harmless to *Aphis rumicis*.

The following plants showed definite activity as contact insecticides: *Mundulea suberosa* Benth. from India; *Neorautanenia fisifolia* (Benth.) C.A.Sm. (= *Rhynchosia fisifolia* Benth.) from Southern Rhodesia; *Tephrosia macropoda* Harv. from Natal. Further experiments were therefore made with these species, and are reported here.

TEPHROSIA MACROPODA Harv.

The genus *Tephrosia* of the Leguminosae includes several species utilised in many parts of the world as fish poisons and, as already mentioned, some are also insecticidal. *T. macropoda* is the best known fish-poison plant in South Africa, and goes by the native name of "ilozane." The following particulars with regard to this plant are given by Howes (3) in a paper on fish-poison plants:

"It is common in the coastal grassveld of Natal and extends irregularly over the greater part of South-East Africa. The plant, which is of a more or less trailing habit, is characterised by a somewhat fleshy variously shaped rootstock. It is this portion of the plant that is used, being merely mashed between stones at the side of a pool or stream before use by the Zulus and other tribes. As legislation now exists against its use in Natal it is not employed as much as it was formerly. An infusion of the roots with water was commonly used by settlers in the early days in Natal as a wash for freeing dogs of fleas and ticks."

Mr W. V. Blewett kindly forwarded samples of *T. macropoda* from Natal some years ago and a note of some preliminary experiments with extracts of the roots and stems as *stomach* poisons has been published (4). A very strong repellent action to young larvae of the winter moth (*Cheimatobia brumata* L.) was observed.

Experiments were made on the action of alcohol extracts of various parts of the plant as contact insecticides in 1926 and 1927, using the black bean aphis, *Aphis rumicis*, under the conditions to which reference has been made. Similar contact experiments were also carried out with larvae of the moths *Selenia tetralunaria* and *Orgyia antiqua* L., the same apparatus and general technique being adopted as with *Aphis rumicis.* After spraying, each lot of ten larvae were immediately transferred to a separate cage with ample fresh food plant, and kept under observation for several days. The data obtained in both sets of tests are set out in Tables I and II.

Table I.

Toxicity of alcohol extracts of Tephrosia macropoda *Harv.*
to Aphis rumicis *L.*

[N = not affected; S = slightly affected; M = moribund; D = dead.]

Date	Material	Concentration: % of plant material	N %	S %	M %	D %	M and D %
1926							
July 29	Roots and stems	1·0	—	—	10	90	100
		0·25	—	—	20	80	100
	Control (0·5 % saponin)	—	95	—	5	—	5
Aug. 25	Leaves	1·0	50	10	20	20	40
		0·2	90	—	—	10	10
	Stems	1·0	—	—	20	80	100
		0·2	30	30	20	20	40
	Control (0·5 % saponin)	—	100	—	—	—	0
Aug. 28	Roots	0·5	—	—	90	10	100
		0·25	10	—	50	40	90
		0·1	20	10	70	—	70
		0·05	50	20	30	—	30
		0·025	90	—	10	—	10
	Stems	1·0	—	—	50	50	100
		0·75	—	—	50	50	100
		0·5	—	—	60	40	100
		0·25	—	30	40	30	70
		0·1	—	50	50	—	50
	Controls (0·5 % saponin and 5–10 % alcohol)	—	100	—	—	—	0
1927							
June 9	Roots and stems	0·5	—	—	40	60	100
		0·25	—	—	60	40	100
		0·1	20	30	30	20	50
June 24	Roots: very roughly ground	0·5	—	—	80	20	100
		0·25	—	10	70	20	90
		0·1	80	20	—	—	0
	Stems: very roughly ground	0·5	100	—	—	—	0
		0·25	100	—	—	—	0
		0·1	80	20	—	—	0
	Control (0·5 % saponin)	—	80	10	5	5	10

The counts were made two days after spraying.

Table II.

Toxicity of alcohol extracts of Tephrosia macropoda *Harv. to larvae of* Selenia tetralunaria *Hufn. and* Orgyia antiqua *L.*

[N = not affected; A = affected; M = moribund; D = dead.]

Material	Concentration: % of plant material	Larvae	N	A	M	D	Feeding and growth
Roots (old extract)	1·0	*S. tetralunaria* (young)	—	—	1	9	None
	0·5	—	—	2	—	7	Very slight
Control (0·25 % soap solution)	—	—	10	—	—	—	Normal
Control (unsprayed)	—	—	20	—	—	—	Normal
Roots (old extract)	1·0	*S. tetralunaria* (about ¾ grown)	—	3	2	5	Slight
	0·5	—	—	7	—	3	Appreciable
Roots (fresh extract)	1·0	—	—	3	6	1	Very slight
	0·5	—	—	9	—	1	Considerable
Control (0·25 % soap + 20 % alcohol)	—	—	20	—	—	—	Normal
Control (unsprayed)	—	—	20	—	—	—	Normal
Roots (fresh extract)	1·0	*O. antiqua* (about 1 month old)	—	3	—	6	Very slight
	0·5	—	—	2	1	7	Very slight
	0·25	—	—	4	—	6	Appreciable
Controls (0·25 % soap solution)	—	—	18	—	—	1	Normal
Controls (unsprayed)	—	—	20	—	—	—	Normal

Final counts made 7 days after spraying. Larvae in column A were in a semi-paralysed condition and it is improbable that they would have completed development. Those in column D were killed almost immediately by the spray.

It is apparent that alcohol extracts of the roots, and, to a less extent, of the stems of *Tephrosia macropoda* possess considerable contact insecticidal properties. The leaves, on the other hand, are of little value, from this point of view. There is some discrepancy between the figures obtained at different dates (see Table I), but this is probably accounted for by less complete extraction of the active principles in the 1927 experiments when the samples were only roughly ground. Moreover, it was not found very easy to separate root and stem exactly, and some of the toxic effect of the stems in the earlier tests may have been due to the inclusion of a proportion of roots. The roots are undoubtedly very toxic and warrant further consideration as a possible insecticide. The plant is apparently common in certain areas in Natal and could presumably be cultivated without great difficulty.

Mundulea suberosa Benth.

This is a common leguminous plant occurring in most parts of tropical and sub-tropical Africa, in Madagascar, and in India and Ceylon. It has long been cultivated and the seeds and bark used as fish poisons (see Howes, *loc. cit.*). It is said to be very rapid and potent in its action on fish. Roark (5) reports that the seeds and inner layer of bark are used both as fish poisons and insecticides in India; and he includes this species (6) in a list of plants, other than Derris, which contain rotenone.

Two samples of *M. suberosa* were received, and differed very markedly in the effect of their extracts on *Aphis rumicis*. Specimens from South Africa sent by the Bureau of Plant Industry, Pretoria, which included stems, bark, cork and leaves, proved to have no appreciable toxicity. On the other hand, the stems, seeds and pods of another sample, from India, forwarded by the Divisional Forest Officer, Dharwar, Bijapur, were quite toxic as the figures in Table III show; the roots and leaves however had no appreciable action at a concentration equivalent to 1 per cent. of the plant material. The stems were the most active part of the plant. The action on the aphides was somewhat delayed; it began with partial paralysis which gradually deepened until the insects became moribund and finally died. The counts given were taken 3 days after spraying.

Table III.

Toxicity of alcoholic extracts of stems, seeds, and pods of Mundulea suberosa *Benth. to* Aphis rumicis *L.*

Concentration: % of plant material	% insects moribund and dead
1·0	100
0·5	100
0·25	20

These figures, while showing the plant to be toxic, do not suggest that it would take a very high place among fish-poison plants as an insecticide. Nevertheless, if, as seems to be the case, it is easy to grow, and if harvesting of the valuable parts of the plant does not present difficulties, the cultivation of *Mundulea suberosa* might prove to be an economic proposition in certain tropical areas.

NEORAUTANENIA FISIFOLIA (BENTH.) C.A.SM. (= *RHYNCHOSIA FISIFOLIA* BENTH.).

This is another leguminous plant which occurs in various parts of eastern South Africa, particularly the Transvaal and Natal, and in Southern Rhodesia. Specimens of the large tuberous roots were received from Mr H. C. Arnold of the Division of Plant Industry, Department of Agriculture of Southern Rhodesia, who states that the natives use them, when mixed with the roots of another legume, *Dolichos lupiniflorus*[1], as fish poisons. He writes: "The two kinds of roots are thoroughly pounded and mixed, mud is added and the mixture thrown into the pool of water which is vigorously stirred. After an hour or two the fish rise to the surface in an intoxicated condition and many of them die within 24 hours." Mr Arnold also reports that an infusion prepared by soaking the powdered root in water for a few days was found to kill bed-bugs and the larvae of the maize stalk borer (*Busseola fusca* Fuller) after momentary immersion. The infusion was harmless to the foliage of maize plants.

Both alcoholic and aqueous extracts of the ground roots diluted with 0·5 per cent. saponin solution were tested and proved to be toxic to *A. rumicis*. Table IV shows the results obtained with the alcohol extract. Control experiments with 0·5 per cent. solutions of saponin containing 1·0, 2·5, 5·0 and 10 per cent. of alcohol showed an average mortality of 10 per cent. of the insects sprayed (maximum, in one test only, 20 per cent.).

Table IV.

Toxicity of alcoholic extracts of tuberous roots of Neorautanenia fisifolia *C.A.Sm. to* Aphis rumicis *L.*

Concentration: % of plant material	% insects moribund and dead
1·0	100
0·5	80
0·25	70
0·1	20

The toxic action of these extracts (as with those of *Mundulea suberosa*) was rather delayed, insects only slightly affected at certain concentrations gradually sinking into a moribund state 12 or 15 hours after spraying. The final counts were taken after 3 days.

It is evident from these figures that the roots of *Neorautanenia fisifolia* possess quite considerable insecticidal properties and would be worth further study with a view to use locally where the plant is readily obtainable

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[1] The roots of *D. lupiniflorus* were also examined but showed no toxicity to *A. rumicis* see list on p. 254.

BLACK HAIARI (*LONCHOCARPUS* SP.).

Data have been previously published with regard to the high toxicity to *Aphis rumicis* of extracts of the stems of black Haiari (*Lonchocarpus* sp.) as contact insecticides (1), and to their strongly repellent action to the larvae of several species of moths, when tested as stomach insecticides (4). As supplementary to these experiments, the results of a small number of tests on the contact insecticidal action of these extracts on caterpillars are of interest and are recorded in Table V. The method adopted for these tests has been referred to in the section dealing with *Tephrosia macropoda*.

Table V.

Toxicity of alcoholic extracts of black Haiari to larvae of Selenia tetralunaria *Hufn. and* Orgyia antiqua *L.*

[N = not affected; A = affected; M = moribund; D = dead.]

Material	Concentration: % of plant material	Larvae	N	A	M	D	Feeding and growth
Black Haiari stems (old extract)	1·0	*S. tetralunaria* (young)	—	4	1	4	Slight
	0·5	—	—	8	—	2	Slight
Black Haiari stems (fresh extract)	1·0	—	—	—	—	10	None
	0·5	—	—	—	—	10	None
Control (0·25 % soap solution)	—	—	10	—	—	—	Normal
Control (unsprayed)	—	—	20	—	—	—	Normal
Black Haiari stems (extract 14 days old)	1·0	*S. tetralunaria* (about ¾ grown)	9	—	—	—	Considerable
	0·5	—	10	—	—	—	Almost normal
	0·25	—	10	—	—	—	Normal
Black Haiari stems (fresh extract)	0·5	—	—	7	—	2	Appreciable
	0·25	—	—	10	—	—	Considerable
Control (0·25 % soap solution and 20 % alcohol)	—	—	20	—	—	—	Normal
Control (unsprayed)	—	—	20	—	—	—	Normal
Black Haiari stems	1·0	*O. antiqua* (about 1 month old)	4	2	1	3	Considerable
	0·5	—	1	4	—	5	Considerable
	0·25	—	2	3	1	4	Considerable
Control (0·25 % soap solution)	—	—	18	—	—	1	Normal
Control (unsprayed)	—	—	20	—	—	—	Normal

Final counts made 7 days after spraying.

It will be seen from Table V, that extracts of this plant are toxic as contact insecticides to larvae of the two species used[1]. The toxicity of the extracts is indeed somewhat greater than is apparent, for the insects included in column A in the table were in all cases partially paralysed and capable of but little feeding; it is very unlikely that any of these would have completed development. Insects in column D were all killed almost immediately by the spray. The figures also indicate that older larvae are much more resistant than young ones to the effect of the extracts and suggest the importance of early spraying if plant insecticides of this type are used against caterpillars. It appears further that alcoholic extracts may lose a proportion of their toxicity if kept for some months.

CONCLUSIONS.

The experiments with fish-poison plants which have been described indicate that although some of these plants have a relatively powerful insecticidal action, such properties are by no means always correlated with a stupefying or poisonous action upon fish. Nor is this to be expected, since compounds such as tannins, saponin and even sugars may be lethal to fish but are in general harmless to insects. Time has not been available for any attempt to separate the active principles from those plants which proved toxic to insects, but it seems very probable that they belong to the class of substances of which rotenone is the characteristic and best known member.

It is interesting that all the plants so far examined which possess both insecticidal and fish-stupefying properties belong to the natural order Leguminosae and a more complete survey of plants of this order may be expected to bring further examples to light. The data recorded here and in previous papers are perhaps sufficient to indicate that there is a wide field of work, likely to produce results of economic importance, in the search for possible new insecticidal plants and in the more detailed investigation of the range of activity and usefulness of those already known.

SUMMARY.

1. Preliminary data is reported as to the insecticidal properties of three tropical fish-poison plants (*Tephrosia macropoda* Harv., *Mundulea suberosa* Benth. and *Neorautanenia* (*Rhynchosia*) *fisifolia* C.A.Sm.).

2. A list is given of other plants (most of them known to be fish

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[1] A single experiment with an old extract of black Haiari stems, using young larvae of *Taeniocampa gothica* L., a Noctuid moth, indicated that this species is highly resistant.

poisons) from many different countries, which have been tested but appear to have little or no toxicity to *Aphis rumicis* L.

3. Extracts of the stems of black Haiari (*Lonchocarpus* sp.) are shown to be toxic as contact insecticides to young larvae of two species of moths. Older larvae are much more resistant.

4. All the plants so far tested which are toxic both to fish and to insects are members of the natural order Leguminosae.

Sincere thanks are due to the agricultural and botanical officers in many different parts of the Empire who have been to much trouble to collect and forward a large number of interesting plants. The authors also desire to express their indebtedness to the Director and other members of the staff of the Royal Botanic Gardens, Kew, through whom much of the material was received, and who kindly supplied much valuable information about individual species of plants.

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The Morphology and Cytology of Bacterium malvacearum, *E.F.S.* *Part* II.—*Reproduction and Cell-Fusion.*

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[PLATES 1 AND 2.]

In an earlier paper (Stoughton, 1929) an account was given of certain observations on the morphological and cytological changes undergone by *Bacterium (Pseudomonas) malvacearum*, E.F.S., the causal organism of the angular leaf-spot disease of cotton plants.

A central, deeply-staining body was demonstrated in the bacterium, and by means of a special staining technique the changes through which it passes were traced. The structure divides simultaneously with the division of the cell-body and the method of this division was described. Further evidence was adduced tending to show that this body is of the nature of a true bacterial nucleus or, alternatively, a nucleus embedded in a matrix of "chromatic" material. The formation and liberation of very small deeply-staining bodies, which appear to be identical with the "gonidia" of other workers, were described.

An account was also given of the method of production of spherical coccus-like bodies, formed by a process analogous to the budding of yeasts, which appear to constitute a method of vegetative reproduction not previously described for the organism. At the time the previous paper was written the subsequent development of these "cocci" had not been traced, but further work has thrown light on this point.

Throughout the work a single strain of *B. malvacearum* of proved virulence has been used. The strain has been kept in a state of assured purity by constant re-plating and occasional re-starting from a single cell isolated by means of the Dickinson micro-isolator (1926). The cultures have been grown throughout on potato-extract agar containing 1 per cent. of sucrose and their virulence maintained by frequent passage through cotton plants. All cultures were kept for at least 1 week in an incubator at 25° C. and then transferred to an unheated storage chamber.

The technique employed in preparing the slides was given in the previous paper, but further work with the method has shown certain points worthy of note. A common source of failure has been the use of too much dye solution in preparing the film of stain. Experience alone will show how much stain is necessary for a good result with any particular organism or culture, but the amount will always be small. The film should be barely perceptible when held to the light, and the final preparation should show the nuclear-like bodies strongly stained and the outer membrane distinct, but the remainder of the cell almost unstained. A second source of failure is the use of too thick a water-film. Using cover-glasses of 2 cm. diameter a loopful of water from a 2 mm. platinum loop just fills the space between the cover and the slide without excess, forming a film approximately 5–10 microns thick. One other point is that slides prepared by this method are in no sense really permanent, but remain good for about 2 days only. After this period granulation of the contents of the cells occurs and entirely misleading appearances may be seen.

For routine examination of the slides a high-power water-immersion objective is a convenience as the covers are not soiled and, if necessary, the slides may be examined repeatedly during the day or two that they remain good. Such an objective is also more suited to the examination of objects mounted in a watery fluid than an oil-immersion lens. For photography, or for critical examination of the finer details an objective with higher aperture and consequent greater resolving power is needed. In this case better results are obtained with the organisms which are in contact with the cover-glass than with those lying on the slide and viewed through the water-film. The latter interferes with the corrections of the objective, though some improvement may be effected by suitable extension of the tube-length. If, however, the film exceeds about 10 μ in thickness, a satisfactory image of the organisms on the surface of the slide cannot be obtained with an oil-immersion lens. The presence of the water-film reduces the working aperture in any case to a maximum of about 1·3, but this is sufficient for a clear resolution of the main details.

Life-cycle of the Cocci.

Certain stages in the production of the coccoid bodies were described in the previous paper, but fuller details of the earlier stages and of the subsequent development have now been obtained. Figs. 1–3, Plate 1, show the first stages in the formation of the bud, before the division of the nucleus in the parent cell. Fig. 4 on the same plate shows the beginning of this division, the later stages of which were given in figs. 3 and 4, Plate 26, of the previous paper. Owing to the optical limitations discussed in the earlier paper and the considerations referred to above, the minute details of the division cannot be finally determined. The process appears to be a "pinching in two" of the "chromatin" material more or less coincidently with the abscission of the neck joining the coccus to the parent rod. In fig. 5, Plate 1, the coccoid body has attained its full size and is on the point of liberation. The coccus now becomes free in the medium, and in a suitable culture large numbers of the free cocci can be seen, each with its single deeply-stained nucleus-like body. These coccus forms are extremely thin-walled, especially while still growing attached to the parent cell, and in consequence are very easily distorted or destroyed. This fact explains the failure to see the structures in dried-film preparations. Even in a well-stained wet-film preparation critical optical conditions are essential for a clear picture of the formation.

After an interval, as yet undetermined, the cocci germinate. A small papilla appears at one point and this grows out into a rod, apparently identical with the normal vegetative cell, figs. 6, 7 and 8, Plate 1. So far, the cytological changes associated with the germination have not been determined. In this stage the cell appears to contain a large amount of food-material which stains rapidly and deeply, the dense stain rendering it difficult to make out the structure. The behaviour is similar in this respect to that of normal vegetative cells from a very young culture, where, as noted in the previous paper, the dense staining rapidly obscures the internal structures.

The development of these bodies seems, therefore, to follow a closed cycle comparable with the vegetative spore-cycle of the lower fungi.

Cell-fusion and "Zygospore" Formation.

In the previous paper reference was made to the occurrence in old cultures (3–6 weeks or more) of characteristic "angled" forms, consisting of two cells apparently united at one end and forming an obtuse angle to one another, fig. 9, Plate 1. It was suggested at that time that this formation might possibly represent an incomplete but more or less normal vegetative division, but further observations have failed to confirm this, and indicate rather that the appearance represents the first stage in the fusion of two independent cells uniting by their extreme ends. A number of forms have been repeatedly observed which fit into a series interpretable as stages in the production of a fusion-cell or "zygospore," and which are difficult to explain on any other basis.

At times the pairs are united by an unmistakable bridge or neck of variable length, fig. 9, Plate 1, but in most cases an obvious tube is not present, and the connection appears to be formed by a breaking-down of the wall of each cell at the point of contact. Here a small swelling appears, very similar in its early stages to the coccoid bodies previously described, fig. 10, Plate 2. This protuberance is at first very thin-walled, and stains only lightly, but at once begins to thicken its walls; the contents become denser and more deeply staining, while, usually, the parent cells become distinctly less dense. The newly-formed body attains a diameter equal to, or rather greater than, the width of the parent cells, figs. 12, 13, 14 and 15, Plate 2. The whole structure stains deeply at this stage, having, as a rule, considerably more affinity for the stain than the vegetative rods. Here again the cytological processes are difficult to determine. In the early stages, where the cells are joined at the tip, the two "nuclear" bodies of the joined cells lie near the point of junction, fig. 10, Plate 2, but in the later stages it is often possible to see the "nuclei" in the middle of each of the subtending cells, fig. 12, Plate 2.

The subsequent history of these spherical spore-like bodies has not yet been determined with certainty. The frequent appearance of two unequal "arms" in the fully-formed structure, fig. 16, Plate 2, where one "arm" seems to be undergoing a process of degeneration, suggests that after the structure has attained its full size the parent cells shrivel and fall off. This inequality of the "arms" was at first thought to be due to the production of the spore-like body from the fusion of two cells of originally different sizes, but in all cases so far observed, when the "fusion-cell" is not full-grown, the subtending cells are equal in size, indicating that the difference is due to subsequent unequal shrivelling of the parent cells. In fig. 15, *b*, Plate 2, the parent cells are shrivelling simultaneously. The "zygospores" when free are similar in size and shape to the vegetatively-produced "cocci," but may in some preparations be distinguished by their affinity for the stain. The fusion-bodies during the course of their development acquire a power of staining strongly and appear as dense spherical cells in which no structure is visible, while the cocci usually stain much more lightly, and this distinction is maintained after liberation. When the coccoid bodies germinate, however, they also become easily and deeply stained. There appears therefore to be no way of determining by the observation of stained preparations alone whether the fusion-cells or "zygospores" subsequently germinate, since no culture containing "zygospores" but free from cocci has yet been obtained, although the latter may occur in the absence of the former.

Bodies similar to these zygospore-like forms have been observed in other plant-pathogenic bacteria, but their development in these cases has not at present been worked out in any detail. A striking example of such formation is given in fig. 18, Plate 2, which is taken from a culture sent as *B. stewarti* to the writer by Dr. A. J. Riker, of the University of Wisconsin. Several of the zygospore-like bodies are shown in one field. In these the subtending cells are nearly empty of stainable material although, in each, one very small granule, which may represent the nucleus, is discernible.

Many of the appearances observed in *B. malvacearum* closely resemble Mellon's figures of "zygospores" in *B. coli* (Mellon, 1925, 1926, 1927). More recently Stapp and Zycha (1931) have observed similar appearances in dried-film preparations of *B. mycoides*, but have interpreted them as artefacts produced by gross overstaining. That the bodies described for *B. malvacearum* cannot be so considered, has been demonstrated by their recognition in preparations of the living, unstained organisms. Three photomicrographs of such unstained cells are shown in figs. 11 and 17, Plate 2, under dark-ground illumination. Fig. 11 shows the early stage of "zygospore" formation immediately after fusion, while in fig. 17 the body is fully formed. The latter photograph shows the highly refractive nature of the mature "spore," which is associated with its strong affinity for the stain in the wet-film preparations. Immature bodies are less refractive than the parent-cells, fig. 11, *b*, Plate 2, a fact which again agrees with the staining capacity.

Further evidence that these zygospore-like bodies are not artefacts is afforded by preparations made by the standard protozoological methods. Fig. 13, Plate 2, is taken from a cover-glass preparation fixed while still wet in hot Schaudinn's fluid, stained by Heidenhain's iron-hæmatoxylin process and mounted in balsam. The appearance is essentially the same as with the wet-film method.

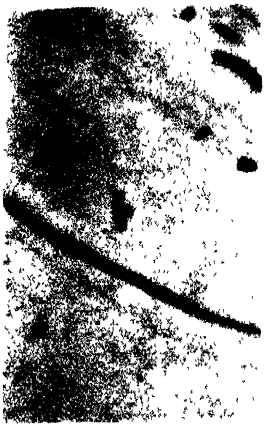
Numerous attempts have been made to watch the various processes occurring under continuous microscopic observation. The difficulties inherent in such work were discussed in the previous paper, and they are reinforced in this case by the time factor involved in the germination of the cocci or "zygospores." Different methods have been tried, including the preparation of minute hanging drops by the use of the Chambers micro-manipulator, the observation of thin liquid films under dark-ground illumination, the hanging-block method, and the agar-film method (Stoughton, 1929). Partial success only has attended these experiments, growth of the rod from germinating cocci having been observed during a few hours. In nearly all cases where the conditions are such that growth can take place, the multiplication of the ordinary rods is so rapid that they soon overgrow the single cell under observation. Further, any interpretations placed on even apparently successful observations should be accepted with great caution, since the optical conditions under which such observations must be made, involving very considerable reduction in the numerical aperture of the illuminating cone, favour the production of false images. The exception would be observation under dark-ground illumination of high obliquity but, as pointed out in the previous paper, this method seems impossible of application in the case of *B. malvacearum*, which requires free access of air for growth.

Summary.

(1) Using a technique described in a previous paper, new morphological forms have been observed in *Bacterium malvacearum*.

(2) The production of coccoid bodies, their liberation, and subsequent germination to form apparently normal rods, are described.

(3) The formation of densely-staining spherical bodies, apparently arising from the point of fusion of two cells, is described. These bodies are apparently liberated by the degeneration of the parent cell.



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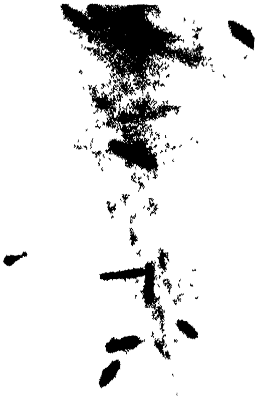

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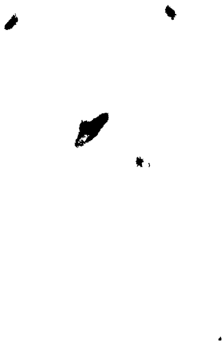

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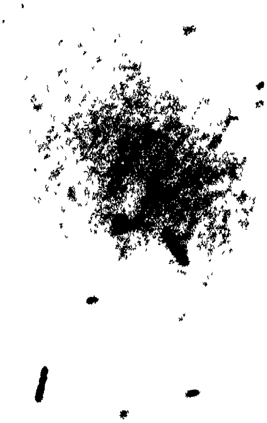

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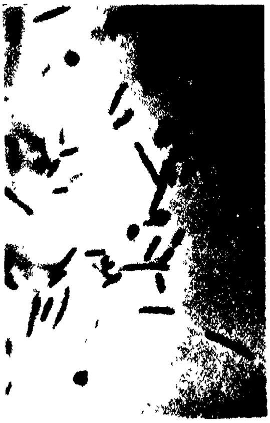

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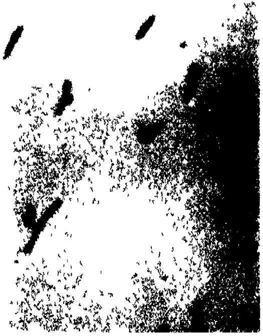

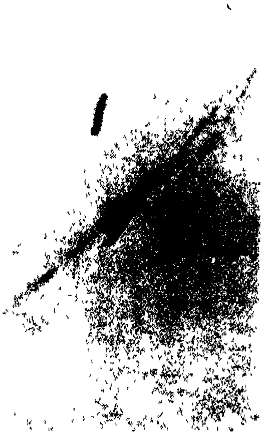

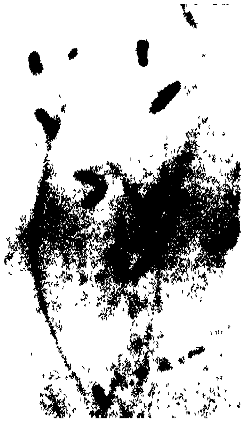

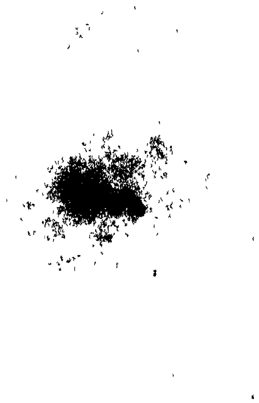

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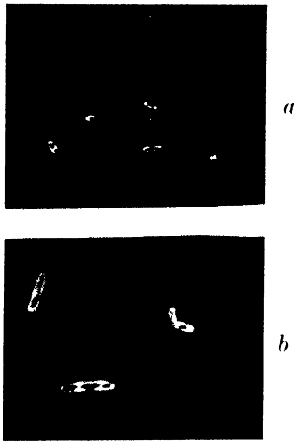

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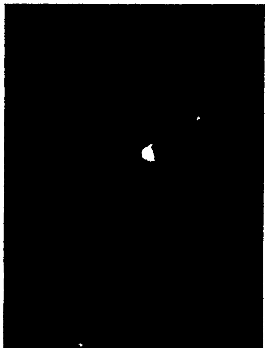

EXPLANATION OF PLATES.

Plate 1.

Figs. 1–5.—4-12 week cultures of *Bacterium malvacearum*, showing stages in the formation of the coccoid reproductive body. Wet-film preparations. Photographed on panchromatic plates with green filter. Leitz 2 mm. fluorite objective N.A. 1·32. × 10 "periplanatic" ocular, Leitz aplanatic condenser, N.A. 1·40. × 2000.

Figs. 6–8.—Stages in the germination of the "cocci" to normal rods. Figs. 6 and 7 photographed with Reichert 1/12-inch achromatic objective, other details as figs. 1–5. × 1600. Fig. 8 as figs. 1–5. × 2000.

Fig. 9.—4-week culture showing early stage of fusion. Photographic details as figs. 1–5. × 2000.

Plate 2.

Fig. 10.—4-week culture showing early stage of formation of zygospore. Photographic details as Plate 1, figs. 1–5. × 2000.

Fig. 11.—5-week culture mounted in sterile water. Photographed on panchromatic plate with green filter. Watson 1/12-inch achromatic objective with funnel stop (N.A. 0·95 approx.), × 10 "periplanatic" ocular, Leitz dark-ground illuminator. (*a*) × 1400, (*b*) × 2200.

Fig. 12.—4-8 week cultures showing formation of zygospores. Photographic details as Plate 1, figs. 1–5. × 2000.

Fig. 13.—4-week culture fixed in Schaudinn's fluid, stained iron-hæmatoxylin. Photographic details as Plate 1, figs. 1–5, *a* and *b*. × 2000.

Fig. 14.—8-week culture showing nearly mature zygospore. Photographic details as Plate 1, figs. 1–5. × 2000.

Fig. 15.—7-week culture showing (*a*) mature zygospore, (*b*) mature zygospore with parent cells beginning to degenerate. Photographic details as Plate 1, figs. 1–5. × 2000.

Fig. 16.—4-week culture showing mature zygospore and degeneration of parent cells. Photographic details as Plate 1, figs. 1–5. × 2000.

Fig. 17.—5-week culture in sterile water. Mature zygospores. Unstained, living forms, dark-ground illumination. Photographic details as fig. 2. × 2200.

Fig. 18.—2-week culture of *B. stewarti* with mature zygospores. × 1500.

N.B.—All preparations are made by the wet-film process except fig. 13, Plate 2, which is a fixed preparation mounted in balsam, and the dark-ground figures, which are water-film preparations unstained.

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THE INFLUENCE OF ENVIRONMENTAL CONDITIONS ON THE DEVELOPMENT OF THE ANGULAR LEAF-SPOT DISEASE OF COTTON

IV. THE INFLUENCE OF ATMOSPHERIC HUMIDITY ON INFECTION

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(With 2 Text-figures.)

EARLIER papers in this series dealt with the influence of soil temperature (2) and of air temperature (5) on the infection of young cotton plants by *Bacterium malvacearum*, the causal organism of the "Angular Leaf-Spot" or "Black-Arm" disease. The experiments were carried out in the Rothamsted control chambers, details of which have been given (1). In these chambers it is possible to control soil temperature, air temperature, air humidity, and illumination, automatically and independently over a wide range. Cotton seedlings make good growth in the chambers, and infection occurs readily under suitable conditions. In the experiments so far described the humidity has in all cases been high, always exceeding 85 per cent. and in some experiments reaching saturation. Work has now been carried out on the influence of different degrees of controlled humidity on the infection of young plants.

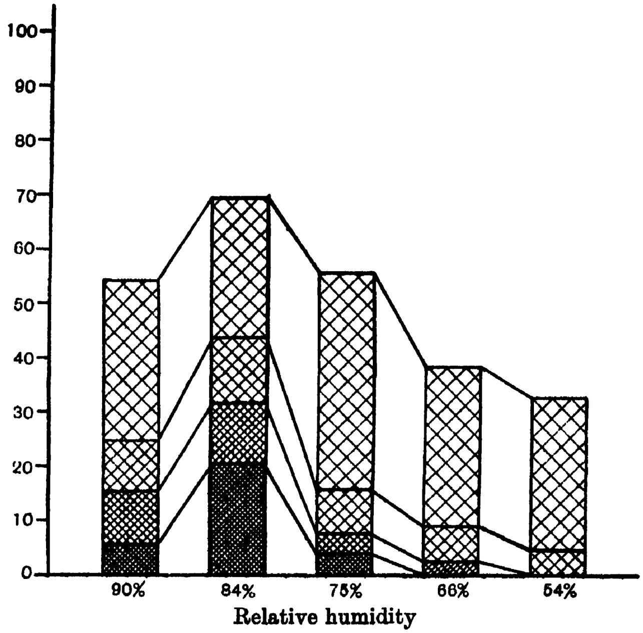
The seed used throughout the experiments has been "Sakellarides" variety from the Gezira Plain, supplied by the courtesy of Mr R. E. Massey, Botanist to the Sudan Government.

DESCRIPTION OF EXPERIMENTS.

Exp. 1. In this experiment, owing to temporary breakdown of one of the control chambers, five of the chambers only were used. The forty soil tins were filled with Gezira cotton soil and sown, in the glasshouse, with Sakel seed, five seeds in each tin. Before sowing the seed was delinted in concentrated sulphuric acid for 10 min., washed and dried, in order to sterilise the outside of the seed. The plants were grown in the glasshouse for 6½ weeks, by which time two true leaves were fully developed, with two or three others unfolding. The five chambers were run for several days before the plants were placed in them in order to obtain settled conditions. The previous experiments had shown that the optimum temperature conditions for infection were about 25–27° C. for the soil temperature and 30–35° C. for the air temperature. The thermostats were therefore set for 25° C. soil temperature and 30° C. air temperature. Good temperature control was obtained in all the chambers throughout the experiments, the range being usually well within 2° C. The humidity controls were set to give average relative humidities of 90, 85, 75, 65 and 55 per cent. A new type of humidifier was in use (4) and the control was quite satisfactory, with a range in most cases not exceeding 5–6 per cent. Owing to sticking of contacts and relays temporary derangements of the controls occurred from time to time, but these were of short duration, and there is no reason to suppose that they could have any appreciable effect on the final result. The lowest humidity proved the most difficult to maintain, and varied at times between 50 and 60 per cent. The average humidity was, however, close to 55 per cent. The actual average humidities were 90, 84, 75, 66 and 54 per cent.

The plants were placed in the chambers for 3 days before inoculation to become acclimatised to the new conditions. They were then sprayed with a strong suspension of *B. malvacearum* in sterile water and left for 1 week, illumination being provided for 16 hours out of the 24. At the end of the first week no sign of infection had developed and it seemed clear that the culture used had lost its virulence. This appears to be a not uncommon phenomenon, and is possibly connected with the dissociation of the organism into strains of varying degrees of virulence, a problem which is engaging attention (3). The plants were accordingly re-sprayed with a new young culture of the organism and left for a further period. Good infection resulted and appeared to be complete in 13 days. At the end of this time the plants were removed from the chambers and the degree of infection estimated.

The difficulty of obtaining a reasonably accurate quantitative estimate of the incidence and degree of infection was considered in the last paper of the series (5), where the method finally adopted was given in detail. Each leaf was examined and the number of disease spots counted, due allowance being made for the cases where spots had coalesced to form a patch, or where an extended lesion occurred along a vein. The results so obtained were then grouped into four arbitrarily delimited classes: Class I, severe infection, fifty spots or more per leaf; Class II, moderate infection, twenty-five spots or more; Class III, light infection, ten spots or more; Class IV, very light infection, less than ten spots. The results of the experiment are given in full in Table I. Where more than about seventy-five spots or the equivalent in patches occurred on a single leaf the infection was recorded as indefinite (∞). Text-fig. 1 shows diagrammatically the distribution of infection in the four classes at the different humidities. It is apparent that the maximum infection occurred at 84 per cent. relative humidity, but it is doubtful whether this is significantly different from the infection at 90 and 75 per cent. humidity. At humidities below these, however, there is a very marked fall in the incidence and severity of infection, the amount of disease becoming very small at a relative humidity of 54 per cent. From Table I it is clear that in accordance with the general rule for this disease the leaves which were unfolding at the time of inoculation (leaves Nos. 4 and 5) are usually the most heavily infected.



Text-fig. 1. Exp. 1. Percentage infection in four classes at various relative humidities.

Exp. 2. This experiment was essentially a repetition of Exp. 1. In order to make the control of humidity easier, especially at the extremes of the range covered, one or two tins in each tank were not used for plants, but were filled, in the case of the higher humidities, with water, or for the lower humidities, with calcium chloride. As in the previous

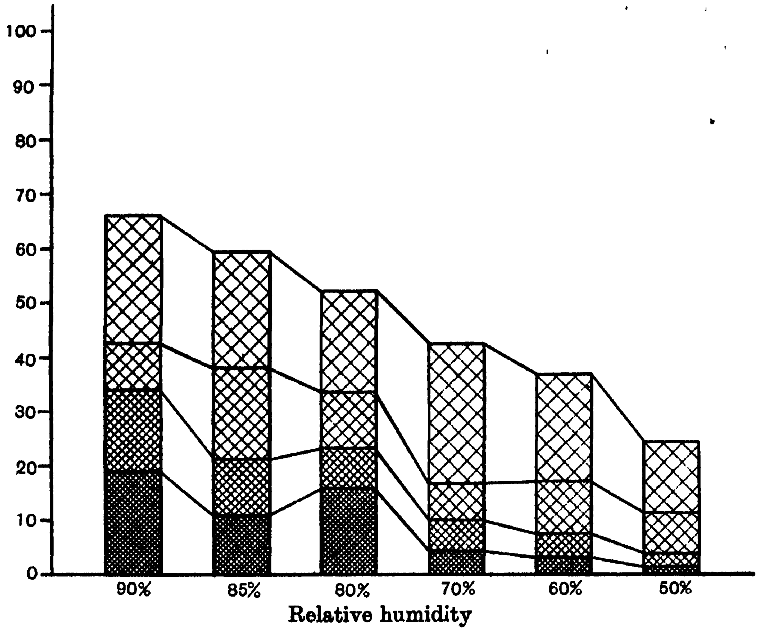
Table I.

Exp. 1. *Distribution of infection at various humidities.*

	90 % Leaf no.						84 % Leaf no.						75 % Leaf no.						66 % Leaf no.						54 % Leaf no.					
	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6
Plant no. 1	—	—	—	17	36	—	—	—	1	2	—	—	—	0	0	4	2	0	—	—	—	0	0	12	—	0	0	0	0	—
,, 2	—	—	1	11	11	0	—	—	0	48	17	—	—	—	1	6	27	—	—	—	1	5	1	—	—	—	0	0	0	0
,, 3	—	—	0	0	0	—	—	—	0	31	∞	8	—	—	0	5	0	0	—	—	0	0	0	—	—	—	0	1	0	0
,, 4	—	—	0	2	21	0	—	—	—	0	77	11	—	—	0	2	29	54	—	—	0	1	0	—	—	0	0	4	1	0
,, 5	—	—	2	35	5	—	—	—	—	3	31	0	—	—	—	7	8	0	—	—	0	0	30	20	—	0	0	6	0	0
,, 6	—	—	1	26	37	0	—	—	—	1	∞	—	—	—	1	0	0	0	—	—	0	2	2	0	—	0	0	0	5	0
,, 7	—	—	0	0	0	0	—	—	—	4	∞	—	—	0	0	1	∞	—	—	—	—	0	0	—	—	0	0	0	0	0
,, 8	—	—	0	0	75	0	—	—	0	52	∞	—	—	—	0	4	17	∞	—	—	0	0	—	—	—	—	0	6	15	0
,, 9	—	—	6	∞	45	5	—	—	2	70	60	0	—	—	—	3	0	0	—	—	0	0	2	0	—	—	0	0	0	2
,, 10	—	—	1	—	37	0	—	—	2	1	24	—	—	—	6	0	0	—	—	—	1	0	0	10	—	—	4	5	0	—
,, 11	—	—	20	6	0	—	—	—	—	12	65	—	—	—	0	3	0	—	—	—	0	0	0	—	—	—	—	15	10	0
,, 12	—	—	11	1	45	0	—	—	—	1	95	0	—	—	—	3	12	0	—	—	3	4	0	—	—	—	—	1	2	0
,, 13	—	—	0	8	0	10	—	—	0	0	45	5	—	—	0	1	0	—	—	—	6	0	0	—	—	—	0	7	5	0
,, 14	—	—	0	50	—	—	—	—	0	0	12	0	—	—	—	6	11	0	—	—	—	0	0	0	—	—	4	0	0	5
,, 15	—	—	0	8	35	—	—	0	0	5	27	6	—	—	2	27	6	0	—	—	0	3	0	2	—	—	0	0	0	0
,, 16	—	—	1	20	0	—	—	—	—	12	0	0	—	—	0	7	27	0	—	—	—	0	0	—	—	—	—	2	0	10
,, 17	—	—	0	12	75	25	—	—	1	9	14	∞	—	—	4	7	0	0	—	—	0	0	0	—	—	—	—	1	6	5
,, 18	—	—	0	0	4	0	—	—	0	41	—	—	—	—	—	7	51	0	—	0	1	0	0	0	—	—	0	9	0	0
,, 19	—	0	2	0	0	—	—	0	4	6	∞	—	—	—	0	3	0	—	—	0	0	0	0	—	—	—	1	0	0	—
,, 20	—	—	2	25	65	—	—	—	0	2	65	0	—	—	0	0	0	—	—	0	1	0	0	—	—	—	—	3	0	—
,, 21	—	—	1	1	0	0	—	—	0	∞	1	1	—	—	—	1	5	0	—	—	2	0	10	—	—	—	—	21	0	—
,, 22	—	0	3	15	—	—	—	—	—	0	1	0	—	—	1	3	7	6	—	—	0	1	0	0	—	—	—	10	0	0
,, 23	—	—	3	2	0	—	—	—	—	13	∞	0	—	—	0	3	0	5	—	—	—	1	1	0	—	—	—	0	0	0
,, 24	—	—	0	0	0	0	—	—	∞	12	∞	0	—	—	0	0	0	—	—	—	—	0	0	5	—	—	10	0	0	—
,, 25	—	—	1	5	5	0	—	—	—	14	∞	—	—	—	4	3	2	5	—	—	—	4	15	1	—	—	5	0	0	0
,, 26	—	—	0	—	60	—	—	—	—	7	0	—	—	—	—	17	15	—	—	—	—	0	2	0	—	—	0	8	5	5
,, 27	—	—	8	2	5	—	—	—	2	36	50	—	—	—	1	4	24	—	—	—	—	0	—	—	—	—	—	0	1	0
,, 28	—	—	4	26	0	—	—	—	—	—	30	—	—	—	—	0	60	—	—	—	—	6	20	—	—	—	0	1	0	0
,, 29	—	—	0	18	5	—	—	—	12	27	0	—	—	—	—	0	5	7	—	—	—	1	20	0	—	—	1	0	0	0
,, 30	—	—	5	—	0	—	—	—	—	35	50	0	—	—	0	7	—	—	—	—	1	0	—	—	—	—	0	1	0	0
,, 31	—	—	0	0	0	5	—	—	—	3	0	0	—	—	—	2	0	0	—	—	3	5	0	0	—	—	2	0	0	—
,, 32	—	—	—	22	65	0	—	—	—	6	∞	70	—	0	0	0	—	—	—	—	5	2	15	—	—	—	0	1	0	0
,, 33	—	0	0	16	0	0	—	—	3	0	0	—	—	—	2	2	19	0	—	—	—	1	35	0	—	—	0	0	0	—
,, 34	—	0	0	0	0	5	—	—	0	13	5	0	—	—	—	12	11	5	—	—	1	5	0	0	—	—	1	1	0	—
,, 35	—	0	0	0	6	—	—	—	—	4	3	—	—	—	0	2	0	—	—	—	0	0	1	0	—	—	—	0	0	0
,, 36	—	—	0	26	∞	0	—	—	60	14	65	48	—	—	0	1	3	1	—	—	0	0	0	0	—	—	2	0	0	—
,, 37	—	—	1	1	3	0	—	—	∞	45	10	—	—	—	0	9	8	10	—	—	0	4	10	0	—	—	0	0	0	0
,, 38	—	—	0	5	0	0	—	—	3	0	0	—	—	—	—	29	0	0	—	—	0	0	0	—	—	—	4	2	0	—
,, 39	—	—	1	8	25	0	—	—	1	35	4	—	—	—	3	3	0	—	—	—	0	7	7	0	—	—	—	—	—	—
,, 40	—	—	2	6	70	0	—	—	—	∞	6	—	—	—	—	0	16	0	—	—	—	—	—	—	—	—	—	—	—	—
Total no. of leaves			142						124						135						125						135			
Class I																														
50 spots and over			8 (5·6 %)						25 (20·2 %)						5 (3·7 %)						0 (0·0 %)						0 (0·0 %)			
Class II																														
25 spots and over			14 (9·9 %)						14 (11·3 %)						5 (3·7 %)						3 (2·4 %)						0 (0·0 %)			
Class III																														
10 spots and over			13 (9·2 %)						15 (12·1 %)						11 (8·2 %)						8 (6·4 %)						6 (4·4 %)			
Class IV																														
Less than 10 spots			42 (29·6 %)						31 (25·0 %)						54 (40 0 %)						37 (29·6 %)						38 (28·1 %)			
Total no. of leaves infected			77 (54·2 %)						86 (69 3 %)						75 (55 5 %)						48 (38 4%)						44 (32·6 %)			

experiment the tins were filled with Gezira soil and sown, in the glasshouse, with Sakel seed, five seeds per tin. The plants were allowed to grow in the glasshouse for 8 weeks, in which time four or five leaves had developed while two or three were still unfolding.

Six chambers were used and the thermostats in all were set for a soil temperature of 29–30° C. and an air temperature of 32–33° C. The average relative humidities in the six chambers were 90, 85, 80, 70, 60 and 50 per cent. The plants were placed in the chambers and left for 3 days to become acclimatised. The period of illumination was as in the previous experiment. On the third day all the plants were sprayed with a suspension in sterile water of a recently isolated culture of *B. malvacearum*, and left for the infection to develop. Symptoms appeared on the fifth day and the infection appeared to be fully developed in eleven days. The plants were removed from the chambers and examined leaf by leaf and the degree of infection estimated by the same method as in the other experiments. The full results are given in Table II, and the distribution of infection in the four classes is shown diagrammatically in Text-fig. 2. It will be seen that the results confirm those of Exp. 1, the decrease in infection with humidity being very regular. The intensity of attack, as distinct from the total percentage number of leaves diseased, is somewhat



Text-fig. 2. Exp. 2. Percentage infection in four classes at various relative humidities.

Table II.

Exp. 2. Distribution of infection at various humidities.

	90 % Leaf no.								85 % Leaf no.								80 % Leaf no.								70 % Leaf no.								60 % Leaf no.								50 % Leaf no.							
	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8
Plant no. 1	—	—	0	7	17	∞	11	—	0	1	0	18	23	0	0	—	0	0	0	0	8	∞	0	—	—	4	1	1	9	20	0	—	—	0	0	1	0	14	∞	∞	0	0	0	3	0	0	—	—
„ 2	—	—	0	43	49	∞	33	3	0	0	0	9	54	1	0	—	—	0	3	27	∞	10	0	—	0	0	1	0	0	—	—	—	—	0	0	0	0	0	40	5	0	0	0	0	0	45	0	—
„ 3	—	—	0	4	∞	∞	45	5	0	0	0	30	46	10	13	—	0	0	0	1	36	∞	10	5	—	0	0	0	0	60	—	—	—	—	0	0	11	20	25	0	0	0	0	0	3	0	—	—
„ 4	—	—	0	2	∞	∞	15	—	0	2	7	∞	∞	—	—	—	0	0	0	1	11	24	∞	—	0	0	0	—	—	—	—	—	—	0	0	0	2	8	58	11	—	0	1	0	15	0	—	—
„ 5	0	0	0	3	4	50	—	—	0	0	0	28	12	1	0	—	0	0	0	0	12	13	—	—	0	0	0	—	—	—	—	—	—	—	0	0	0	24	0	—	0	0	0	0	15	—	—	—
„ 6	—	0	0	0	9	∞	17	—	—	—	0	1	15	11	45	0	—	0	0	0	47	3	1	—	0	0	0	0	0	—	—	—	—	0	2	0	0	18	35	12	—	0	0	0	0	0	0	—
„ 7	—	—	0	0	15	∞	—	—	—	0	7	22	12	—	—	—	0	0	0	∞	∞	10	—	—	—	0	0	4	4	∞	—	—	—	—	0	0	0	20	0	—	0	0	0	1	0	0	—	—
„ 8	—	—	0	3	41	45	∞	—	0	0	3	18	3	—	—	—	—	0	1	0	6	33	∞	6	—	0	0	0	5	9	35	—	—	0	0	0	0	2	40	2	0	0	0	0	—	—	—	—
„ 9	—	—	0	39	∞	11	—	—	—	—	0	0	3	∞	1	—	—	0	0	1	12	14	∞	∞	—	—	0	0	2	13	60	—	—	0	0	0	1	20	—	—	0	0	2	0	—	—	—	—
„ 10	—	—	1	2	∞	30	—	—	—	0	13	12	16	44	1	—	—	0	0	0	19	∞	∞	27	—	1	3	1	∞	25	—	—	—	0	0	0	0	0	6	1	0	0	0	0	0	—	—	—
„ 11	0	0	0	0	21	∞	—	—	0	0	12	27	∞	—	—	—	—	0	0	0	14	∞	∞	—	—	0	0	0	0	0	42	5	—	0	0	0	0	0	—	—	—	—	0	0	0	16	10	—
„ 12	—	—	0	12	38	48	6	—	—	0	—	15	0	—	—	—	—	0	0	0	3	51	17	2	—	1	0	1	∞	35	15	5	0	0	0	0	7	5	—	—	—	0	0	0	0	5	0	—
„ 13	—	0	6	3	40	0	—	—	—	0	6	1	2	11	0	—	—	0	1	0	∞	5	—	—	—	0	0	0	0	20	—	—	0	0	5	5	—	—	—	—	0	0	0	0	—	—	—	—
„ 14	—	0	0	0	1	5	∞	31	—	0	0	16	∞	48	10	—	—	0	0	0	32	—	—	—	0	0	0	1	6	0	—	—	0	0	0	0	0	8	0	—	—	0	0	0	20	—	—	—
„ 15	—	—	0	0	3	∞	∞	35	2	3	6	∞	—	—	—	—	—	0	—	∞	∞	∞	—	—	0	0	0	0	4	1	45	6	—	0	0	1	1	58	∞	—	—	0	0	0	5	0	—	—
„ 16	—	0	0	0	11	—	—	—	0	0	∞	26	0	—	—	—	—	0	0	48	0	—	—	—	—	0	0	0	1	26	10	—	—	—	0	0	5	0	5	0	—	0	0	0	2	17	—	—
„ 17	0	0	0	9	∞	∞	—	—	24	4	∞	47	5	∞	—	—	—	0	33	12	31	1	∞	∞	—	0	0	0	0	5	0	—	—	—	0	0	0	11	0	0	—	0	0	0	—	—	—	—
„ 18	—	0	9	42	∞	6	—	—	0	0	8	24	0	0	—	—	0	0	0	0	7	∞	∞	0	—	—	0	1	0	35	5	—	—	0	0	1	28	—	—	—	—	0	0	5	0	0	—	—
„ 19	0	1	0	4	∞	49	3	—	—	0	0	10	2	33	32	—	—	0	0	1	6	5	∞	10	—	0	0	5	0	—	—	—	0	0	0	0	10	0	—	—	—	—	0	0	1	20	0	0
„ 20	—	0	2	∞	∞	43	15	—	—	0	0	0	10	9	0	0	—	0	0	5	46	2	—	—	—	0	0	0	19	55	∞	—	—	0	0	0	1	5	0	—	—	0	0	0	0	31	—	—
„ 21	—	4	0	14	∞	38	3	—	—	—	0	1	1	9	0	—	0	0	0	0	—	—	—	—	—	0	0	1	10	6	0	—	—	0	0	0	0	5	—	—	0	0	0	0	15	—	—	—
„ 22	—	0	0	8	6	∞	15	0	—	0	—	∞	14	0	—	—	—	0	0	0	15	45	—	—	0	0	0	1	0	0	—	—	—	—	0	0	0	0	0	—	0	0	0	0	2	—	—	—
„ 23	—	0	0	42	∞	∞	∞	—	—	—	—	42	45	∞	0	—	—	—	0	0	1	29	∞	—	0	—	0	6	—	—	—	—	—	0	0	0	13	—	—	—	—	0	0	0	0	1	0	0
„ 24	0	0	37	23	7	—	—	—	—	0	—	6	∞	∞	7	—	0	0	0	40	5	—	—	—	0	0	0	8	1	0	0	—	—	—	0	0	6	15	—	—	0	0	0	0	0	∞	—	—
„ 25	0	0	1	∞	38	0	—	—	—	1	1	36	21	0	—	—	0	0	0	∞	∞	—	—	—	—	0	0	10	12	48	—	—	—	—	0	0	0	16	14	11	0	0	0	1	5	6	—	—
„ 26	0	0	0	46	1	—	—	—	0	4	0	17	30	0	—	—	0	0	6	∞	5	—	—	—	0	0	0	1	0	12	—	—	—	—	0	0	42	—	—	—	—	—	0	0	13	∞	—	—
„ 27	—	0	0	13	∞	∞	13	5	—	0	3	3	23	∞	25	—	—	6	1	∞	5	1	—	—	0	0	0	1	6	0	0	—	—	—	0	0	3	1	5	—	—	0	0	1	15	15	—	—
„ 28	—	0	2	0	48	∞	27	30	—	0	0	0	∞	∞	0	—	—	—	0	0	2	12	0	—	—	0	0	3	0	2	—	—	—	—	—	1	1	0	40	0	—	0	0	0	0	12	30	—
„ 29	0	0	0	45	∞	∞	9	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0	0	4	40	15	—	—	—	—	—	—	—	—	—	—	—	—	0	0	0	1	28	—
„ 30	—	1	2	61	∞	32	5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0	0	1	0	0	—
„ 31	0	0	1	∞	9	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Total no. of leaves	175								155								163								160								158								157							
Class I 50 spots and over	33 (18·9 %)								17 (11·0 %)								26 (16·0 %)								7 (4·4 %)								5 (3·2 %)								2 (1·3 %)							
Class II 25 spots and over	26 (14·8 %)								16 (10·3 %)								12 (7·4 %)								9 (5·6 %)								7 (4·4 %)								4 (2·5 %)							
Class III 10 spots and over	15 (8·6 %)								26 (16·8 %)								17 (10·4 %)								11 (6·9 %)								15 (9·5 %)								12 (7·6 %)							
Class IV Less than 10 spots	41 (23·4 %)								33 (21·3 %)								30 (18·4 %)								39 (24·4 %)								31 (19·6 %)								20 (12·7 %)							
Total no. of leaves infected	115 (65·9 %)								92 (59·4 %)								85 (52·1 %)								68 (42·5 %)								58 (36·8 %)								38 (24·2 %)							

greater than in Exp. 1, corresponding with the slightly higher air temperature employed. This second experiment indicates also that the apparent decrease in infection at the highest humidity observed in the previous case was not significant.

Under constant conditions, therefore, it appears that infection occurs most readily when a high air temperature is coupled with a high humidity, and a decrease in either of these factors reduces the amount of disease. The methods of action of the two factors are, however, entirely different in nature. In the previous paper the theory was advanced that a high air temperature acts indirectly on the parasite by affecting the rate of maturation of the host, and possibly by altering its carbohydrate metabolism, with a resulting increase in the sugar content of the leaves. It is probable on the other hand that the importance of humidity is mainly physical in nature. The bacteria are motile, and gain access to the tissues of the leaf through the stomata. The method of inoculation adopted of spraying the leaves with an emulsion of the organisms deposits the bacteria in innumerable droplets of water on the leaf surface, and each droplet will cover a number of stomata. If the droplet persists until the bacteria have entered the stomata a lesion will result provided the other conditions are favourable. The average time the droplets will remain depends directly upon their size and upon the humidity of the atmosphere, so that the chances of infection occurring are greater with a high than with a low humidity. Even at low humidities, however, some drops of larger size will persist for a sufficiently long time for penetration of the stomata to be effected. Local variations in the humidity will also occur, and in some cases leaves will stick together while wet, with the result that some leaves become severely infected even at these low humidities. That humidity is of importance only up to the time when penetration of the stomata is achieved by the bacteria is shown by the fact that in experimental inoculations in the glasshouse, infection rarely occurs from spray inoculations, while a lesion invariably results from prick or injection inoculations. The problem of the time during which a high humidity must persist will be discussed in a later paper on the subject of alternating and regularly fluctuating conditions.

SUMMARY.

Experiments carried out in the Rothamsted control chambers on the influence of atmospheric humidity on the angular leaf-spot disease of cotton, resulting from spray inoculation of young plants, show that high humidities favour the development of the disease. Maximum infection

occurs at humidities exceeding 85 per cent. and at humidities below this the degree of infection decreases rapidly.

The relation of these results to the experiments on the influence of air temperature is discussed, and it is concluded that the importance of humidity is mainly physical in nature, by affecting the time during which the infection droplets persist.

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THE FORMATION OF INTRACELLULAR INCLUSIONS IN SOLANACEOUS HOSTS INFECTED WITH AUCUBA MOSAIC OF TOMATO[1]

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(With Plates XXV–XXXIII.)

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INTRODUCTION.

MANY virus diseases of plants and animals produce abnormal inclusion bodies in the cells of the host. These were first observed in plants by Iwanowski (10), who described the inclusions which appear when tobacco is infected with mosaic disease. His observations have since been confirmed by a number of workers (2, 3, 4, 6 and 19), but the phenomena observed have been interpreted in varying ways. Similar cytological effects are caused by this disease in other hosts (8), and intracellular inclusions are produced in the host plant by many other virus diseases such as curly top disease of sugar beet (18), dahlia mosaic (7) and virus diseases of potato (8, 25). They are formed also in the cells of diseased monocotyledonous plants such as sugar cane infected with mosaic disease (2, 14), in wheat with rosette disease or with a mosaic-like leaf mottling (16) and in Hippeastrum mosaic (9, 13).

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When aucuba mosaic of tomato is inoculated into *Solanum nodiflorum* large conspicuous protein bodies are produced in many of the cells of the host plant (24). A short note as to their mode of origin has been given by the writer and J. Henderson Smith (21). It is proposed now to give a fuller description of the development of these intracellular inclusions and also to compare the reaction of other hosts to this virus.

MATERIAL AND METHODS.

The main part of the paper concerns *Solanum nodiflorum* infected with aucuba mosaic of tomato (22). *S. nodiflorum* is an exotic species closely related to the British forms *S. nigrum* and *S. dulcamara*. It is of no economic importance, but has been selected for intensive study as it shows some very clean cut cytological effects. The action of the disease was studied also in the cells of several other Solanaceous hosts, viz. *S. nigrum* (black nightshade), *S. lycopersicum* (tomato), *Nicotiana tabacum* (tobacco), and *Hyoscyamus niger* (henbane). The plants were grown in insect-proof glasshouses. Seedlings were inoculated with the virus by leaf mutilation.

Fixing and staining methods.

An attempt was first made to study the cell contents of leaves of *S. nodiflorum* from microtome sections of fixed material. A large number of fixatives were tried, including chrome-acetic mixtures, Flemming's fluids and several of their modifications, Bouin and Allen's modified Bouin, Zenker and Helly's modification, Worcester, Gilson, etc., as well as mitochondrial fixatives such as Altmann, Benda, Regaud, Champy and the osmic impregnation methods described by Bowen (1). Very little success was attained by any of these methods. It is sometimes possible to fix fully formed inclusion bodies without their being greatly distorted, but in the earlier stages of development of the disease the contraction of the cytoplasm due to poor fixation obscures the effects of the virus. The cells of the palisade tissue, epidermis and especially of the hairs of the leaves of this species are all very vacuolate; moreover, the cell walls are rather thick and the epidermis is cuticularised. Also the hairs tend to hold the air and prevent the fixing agent from reaching the epidermis. Consequently if a fixative is sufficiently strong to penetrate the tissues rapidly, it is impossible to avoid plasmolysis within the cells. Weaker fixatives are unable to penetrate the protective layers before the cell contents show signs of death. To assist penetration the leaves were cut up into small portions and sometimes the epidermis was stripped from one surface. If before fixing in one of the weaker fluids the tissues are immersed for a short time in absolute alcohol, chloroform or Carnoy's fluid, slightly improved fixation is sometimes obtained. These methods have not yet been extended to the other hosts used where penetration would probably be easier.

Some difficulty was also experienced in finding a differential stain for infected material, both nuclear and cytoplasmic stains being taken up by the inclusion bodies. Feulgen's fuchsin sulphurous acid stain, the only specific stain for chromatin known, was used in combination with a dye such as Orange G, light green, methylene or aniline blue as a counterstain. The inclusion bodies then took on a somewhat deeper colour than the cytoplasm.

As the study of such fixed and stained material gave no very useful data it was abandoned for a time. Such results as were obtained served only to confirm the observation made on living material.

Vital methods.

Hand sections mounted in water or sugar solution, sometimes coloured with a vital stain, were used, observations being made only on uncut cells. Unfortunately, such cells remain in their living condition for only a few minutes. The epidermis can be preserved in the living state for a longer period if it is stripped from the leaf and mounted separately. It was found that a study could be made very simply of the cells of the hairs which cover the leaf surfaces of many Solanaceous plants as the difficulties of technique in the handling of living material are minimised. Also this study was the most productive of results, since intracellular inclusions are formed in these tissues with greater regularity than elsewhere in the plant and usually the cells are comparatively free from other inclusions. The hairs of *S. nodiflorum* are quite stiff, and a row of them project from the leaf margin. If a strip be cut from the edge of the leaf and mounted in water, a single living cell can be kept under observation for some hours. The thick wall which delays penetration by the fixing fluids, now prevents penetration by the mounting medium. In some of the other hosts, these cells are not quite so convenient to work with as the hairs are longer, softer and thinner walled, but always they are simpler to examine in the living condition than are any other tissues.

To study the development of inclusions formed as a result of infection with a virus, a leaf was detached from the plant at a suitable period after inoculation. It was mounted in water on a microscope slide, care being taken to immerse the petiole. The hairs at the margin of the lamina were examined; if they appeared as in a healthy plant, the leaf was removed from the slide and the petiole was immersed in a nutrient solution. At intervals the leaf was remounted and re-examined. In this way it was kept alive for several days, and the whole process of development of intracellular inclusions was traced in the cells.

Attempts were made to stain the cells of the growing plant. A leaf lamina was removed and the cut petiole immediately plunged into a tube containing a solution of a vital stain such as dahlia violet, trypan blue, Janus green, neutral red, Bismarck brown, iodine green or methylene blue. All of these dyes were carried up in the water stream. The cell walls often became stained, also the contents of the glandular hairs, but no dye could be found to penetrate into any of the other cells.

If seedlings are inoculated with a mixture of crude virus juice and the dye, the plants become infected with the virus, but again only the cell walls are stained.

Photographic methods.

It was desirable to demonstrate many of the observations by photomicrographs. It was often impossible to take such photographs by the usual methods as, owing to the rapidity of movement within the cells, the plates of any ordinary photomicrographic apparatus could not be changed sufficiently quickly to take a series of photographs at frequent intervals. Resort was taken to the cinema camera, and the entire process of the formation of virus bodies in *S. nodiflorum* infected with aucuba mosaic was filmed. Many of the figures illustrating this paper are enlargements from single pictures of this cinema film. In some of them detail is ill-defined, not because the object is out of focus but because of its rapid movement. As the illuminant, oxy-acetylene gas with a thorium pastille was used; this illumination was not sufficiently intense to allow the ciné film to be taken at the normal speed of sixteen pictures per second. Actually the exposure given for each picture was twelve times as long as is usual, thus accounting for a lack of definition in moving objects when viewed on a single photograph of the series. However, the consequent speeding up when projected renders evident slow movement which is not discernible visually.

For all microscope work, both visual and photographic, transmitted light was used. If dark ground illumination be used, the thickness of the object causes much of the light to be lost by refraction from its surface and only a very poor image of the cellular contents is obtainable. With transmitted light an appreciable amount of contrast can be obtained between the rather translucent object and the background by closing the iris diaphragm of the substage condenser.

DESCRIPTION.

Solanum nodiflorum and Solanum nigrum.

These two species are closely related and, apart from the obvious morphological differences, the only variation observed was a slightly greater permeability of the cell walls of *S. nigrum*. Otherwise it was impossible to distinguish the two forms histologically or cytologically. They will therefore be treated together.

The normal plant.

The leaf lamina consists of a single layer of closely packed palisade tissue, containing innumerable chloroplasts, towards the upper surface and about four layers of more loosely packed parenchymatous tissue below. The whole is covered by a cuticularised epidermis; many of the epidermal cells, especially those on the upper surface and at the leaf margin proliferate into hairs. Some of these are glandular, but the majority are of the protective type. Each of the latter is horn-shaped, curving round towards the leaf tip. All cell walls are very thick and the whole outer surface of the hair is minutely papillose.

Each hair consists of three or four vertically seriated cells, the lowermost is the largest, the others tapering gradually towards the apex. Each cell is a highly vacuolated structure (Figs. 1, 26). The wall is lined with a very thin layer of cytoplasm in which are embedded innumerable highly refractive particles, mitochondria and oil globules. Plastids occur in the hairs of very young leaves, but are not usually found in older hairs. The plasm flows continuously around the cell carrying these minute inclusions with it. Fine strands of plasm may flow across the central vacuole. Often these are thrown out from the peripheral layer. They may soon break and the torn ends flow again into the parietal plasm. Such strands often anastomose, but they may break apart again quite soon. Sometimes they lie in such close juxtaposition as to appear as a single thread, until on closer examination this is seen to consist of several strands in which the cytoplasm may be flowing in contrary directions. Occasionally the plasm seems to reverse its direction of flow: this may be due to two threads being superimposed, then one breaking away or being lost to view.

Each cell contains a nucleus which is carried passively about the cell by the streaming plasm, but its movement is never so rapid as that of the small inclusions and the plasm which carries them. Often it is spherical, but changes its shape as it is caught in the varying currents of the plasm. It may come to rest for a while against the cell wall when it usually assumes a lenticular form and the cytoplasm then flows around it. However, it is soon carried away again by the plasm. It may be rolled along against the wall or flung out towards the centre of the cell. Sometimes it is suspended in the vacuole by strands of flowing plasm which radiate from it towards the cell wall. Frequently anastomoses occur between threads, but when a large number of strands meet they are usually found to converge upon the nucleus. The cell contents may maintain such a state of equilibrium for a while, but ultimately some of the threads break away and the nucleus collapses against the cell wall or it is again caught up in a stream of flowing plasm. The degree of activity of the cell contents may vary with the temperature and illumination, but they are never actually at rest. Sometimes no movement is discernible by the eye, but even then it can be observed by magnifying the speed of motion through the medium of the cinema camera.

The diseased plant.

For a few days after inoculation no macro- or microscopic differences are observable in the host plant. However, about the fifth day, if growing conditions are good, there is a clearing of the green colour from the region of the veins, the first sign of chlorosis. At about the same time definite symptoms are seen within the hair cells. It is not known how long the virus takes to reach the individual cells, hence we have but little idea how much time elapses between inoculation of the individual cells and their reaction to the virus. The first effect of the virus to become obvious microscopically is an increasing activity of the cell contents. The cytoplasm becomes more conspicuous and may appear to increase in bulk. It flows with increasing rapidity around and about the cell. Thicker threads composed of many closely packed strands, in which the plasm may be flowing in contrary directions, as well as fine threads may appear across the vacuole. As in the healthy plant these are soon again absorbed into the peripheral plasm and fresh ones appear in new places. The nucleus continues to be buffeted by the flowing plasm, and it may be moved about the cell somewhat quicker than before.

The development of intracellular inclusions.

After some time a number of very small particles appear in the streaming cytoplasm (Fig. 2). These are of a faint yellowish colour, are angular and highly refractive. They are carried about the cell, their movement being comparatively rapid. More and more particles appear until they are quite innumerable. The speed at which they travel varies: if illumination or temperature is increased the rate of flow of the cytoplasm becomes greater with a corresponding increase in the speed of movement of the contained particles. Those in the parietal cytoplasm often tend to move more slowly than those in the strands crossing the vacuole. Often a particle halts for a while, but ultimately it is moved on again. Such a temporary cessation of movement occurs most frequently when it reaches the point of convergence of several strands of cytoplasm; at such a junction the particle may stay for a time before it is caught up in one of the diverging currents. Often particles are brought together in this way (Figs. 6 *a* and *b*), and they may fuse together either immediately or they may pass along a thread of plasm together for a while and fuse later. Sometimes one of these particles, after moving steadily along a cytoplasmic strand, will suddenly dart a short distance and then resume its former steady pace; possibly it has encountered a path recently traced through the colloidal plasm by a similar moving particle. In this way one particle may overtake another which is moving more slowly (Figs. 7 *a–e*) and they may unite. Sometimes particles come together for a while only to be caught in diverging currents and drawn apart again. However, fusions are comparatively frequent and the particles become fewer in number and larger in size (Fig. 8). Often a number accumulate about the nucleus, for, always if it is suspended in the cavity and sometimes even if apposed to the cell wall, it is the point of convergence for many of the cytoplasmic strands. It must not be regarded as due to any special property of the nucleus that the cytoplasm surrounding it is often studded with these particles, for they almost always accumulate if a number of strands converge on any one point for an appreciable time. Often particles lie against the nucleus for some hours, then by some new activity of the streaming cytoplasm they are whirled away to be again carried about the cell.

The streaming of the cytoplasm and the consequent movement of the particles is ceaseless. By aggregation and subsequent fusion the particles increase in size (Fig. 9). As they so increase their movement becomes somewhat slower (Figs. 10 *a* and *b*). There is no evidence at any time that it is autonomous: the change in speed is probably partly a mechanical effect due to the increasing mass of the particles. Also the rate of cytoplasmic streaming appears to be gradually decreasing. By this aggregation the number of particles is gradually decreased until after a day or so a few large masses circulate slowly about the cell (Figs. 11–14). These masses are irregular in shape and consist obviously of innumerable loosely packed particles. They are plastic and change their shape as they are carried in the plasmic stream. A few small particles are still circulating freely, but almost all of them are gradually absorbed into the larger masses. The masses become more compact, but occasionally a fairly large portion will break away from the aggregation (Figs. 12, 22). This moves around the cell and ultimately it may again unite with the mass from which it broke away or it may join up with one of the other aggregations of particles.

A number of microchemical tests were made on the particles and on the masses which they later form at all stages of development in the living cell side by side with similar tests on the fully developed inclusion body. The latter have been fully described by Henderson Smith (23) and need not be dealt with in any great detail here. Except when using very strong reagents it is often difficult to be certain that penetration has occurred, but the general results obtained point definitely to a protein nature. Particles of all sizes, and the large aggregations are, like the completely formed inclusion body, insoluble in water, ethyl alcohol, xylol, acetone and chloroform. They are rapidly soluble in 10 per cent. KOH and in strong concentrated mineral acids. With osmic acid a brown coloration is usually obtained, but if material is fixed by the osmic impregnation methods of Bowen a blackening of particles and of the inclusion body occasionally results. However, no reaction was given with Sudan III or IV. Iodine appears to deepen the natural yellow colour of the particles and fully formed body; a similar result is obtained by the xanthoproteic reaction. A very definite protein reaction at all stages is given by Millon's test, the completed inclusion body and the larger aggregations giving a red coloration, whilst the smaller particles assume a somewhat paler tint of red.

The protein masses gradually unite together until practically all the material is contained in a single loosely packed, large and very irregularly shaped body (Figs. 15, 31), portions of which may break away only to rejoin later. The mass soon becomes much more compact, thereby considerably decreasing its volume; it loses its irregular outline, usually becoming roughly spherical. It happens occasionally that all the protein material does not unite into one large mass, but two may be formed which round off independently (Figs. 21, *a–d*). They may fuse later, but usually result in the ultimate formation of two more or less spherical inclusions. The inclusion body, or bodies, are now completely formed. Small portions may still break away, and after a shorter or longer period rejoin the main body. A few very small particles may still be moving about the cell independently of the large inclusion body. Sometimes they apparently join it only to move away again, or they may be absorbed into its substance.

The distribution of these bodies throughout the host plant will be dealt with later, but in whatever tissues of *S. nodiflorum* these bodies have been observed their mode of formation is precisely the same. At this stage the body appears rather granular (Fig. 16), but when it has become compact in many cases it loses this appearance. In the hair cells the granular form is often retained, but is almost always lost in all other tissues. The constituent protein substance becomes homogeneous and vacuoles may appear in it (Fig. 23). In the inclusion bodies of the hairs vacuolation occurs in about one-half the cases, although the granular form may be retained, but in those of other tissues it occurs almost invariably. At no time could any indication of a limiting membrane be found.

If the host plant is kept under good growing conditions the process of formation of the rounded inclusion body occupies two or three days only. The condensation of the protein substance to give a more compact body and the process of vacuolation continues for some days after this. If, however, growing conditions are poor, as during the winter, the development of inclusions is retarded as is the development of macroscopic symptoms. For a long while large irregular masses of protein material move slowly about the cell. Usually they seem less granular than in the more rapidly growing plant, appearing to consist of aggregations of a more crystalline material. Ultimately these masses come together to give the typical inclusion body.

When the inclusion body is fully formed the activity of the cell contents is greatly diminished. Very little cytoplasm is visible; the peripheral lining layer is still present and streams feebly around the cell. Occasionally a few small protein particles are seen in it. Threads of plasm are thrown out across the vacuole, but only very rarely (Fig. 19). The inclusion body may move, but usually its motion is so slow as to be scarcely discernible. Often it settles down against the cell wall, frequently against the lower septum, and may then become somewhat flattened as the nucleus almost invariably does if in a similar position

(Figs. 18, 19, 32). A second inclusion may become evident in some of the hair cells (Fig. 20); this is a long colourless spike-like crystal (24). Its length is one-half to three-quarters that of the cell in which it lies apparently motionless. Occasionally two such crystals are present.

The cell contents now continue practically at rest for the space of several weeks, after which the large spherical body begins to crystallise out. Crystals are formed first on the surface, these move away from the body and gradually the whole mass breaks down. After a while the crystals, which give a strong protein reaction, are dispersed throughout the cell (Fig. 24). Then they gradually decrease in number until after about four or five months no sign is left of them or of any virus inclusion bodies. Presumably these crystals as well as the spike have been dissolved into the cell sap (Figs. 25, 33).

Size and distribution of the intracellular inclusions.

Significance has often been attached to the apparent association of nucleus and inclusion body, as they often lie in close proximity; it has been suggested that the latter might be a degeneration product of the nucleus. However, it has been shown that, owing to the confluence of several strands of cytoplasm in this area, particles tend to accumulate around the nucleus. Consequently the largest aggregations of protein material are often found here, the inclusion body being ultimately formed in close proximity with the cell nucleus. This is by no means a general rule. Any part of the cell may become the focus for the final agglomeration of all the protein particles, but it is usually the point of convergence of several strands of streaming cytoplasm. If the body be formed near the nucleus, it may move away later, or if it be formed independently of the nucleus it may join it later but possibly only for a while. Sometimes during its movement about the cell the body impinges on the nucleus causing a depression to appear in the surface of the latter; if it moves away again the nucleus resumes its former rounded contours. The nucleus itself appears to be totally unaffected by the virus disease.

Although both symptoms appear about the same time, the formation of intracellular inclusions bears no apparent relation to the development of chlorotic areas on the leaf. Bodies are formed in the green tissues equally abundantly as in the yellow tissues. Such bodies are contained by practically every hair of an infected leaf; possibly only the basal part of the leaf may show the mosaic, but the inclusions are equally prevalent in the hairs on the green area towards the tip. The inclusions are always larger than the cell nuclei, but their size varies and probably bears some relation to the size of the containing cell and to the amount of cytoplasm present. The largest inclusions are found in the basal cells of the hairs where they often reach a diameter of 30μ. In the upper cells of the hair they are usually smaller (15–20μ), and they may be entirely absent from the distal cell. Although inclusion bodies occur with such frequency in the protective hairs they have never been observed in the glandular hairs. These each consist of a single stalk cell and a secretory head divided into quadrants. The latter are normally filled with protein material which might possibly obscure any virus inclusion.

In both the upper and the lower epidermis (Figs. 29, 30) inclusion bodies are formed in only a small percentage of the cells, not with nearly such frequency as they occur in the hairs. If one epidermal cell is found to be so affected, all the cells in its vicinity are found to contain such bodies. These patches of affected cells in no way correspond to either the green or the chlorotic areas; they occur indiscriminately in both. In the elongated epidermal cells covering a vein such bodies appear in a somewhat larger proportion of the cells; here again the affected cells are restricted to certain areas. The epidermal inclusion bodies are smaller than those of the hair cells, being only about 10μ in diameter.

In the palisade tissue inclusions are rather rare and when they occur are even smaller than in the epidermis, reaching only about 7μ in diameter. Here again affected cells are restricted to definite areas which, in the instances observed, were always below epidermal cells containing inclusion bodies. Similar bodies have been observed in the parenchymatous tissue below, but only very rarely indeed. So far no inclusions have been observed in vascular tissues.

The spherical inclusions and the spike-like crystals are by far most abundant in the tegumentary tissues, especially those near the vascular bundles.

Solanum lycopersicum.

The normal plant.

The leaf of *S. lycopersicum* is a more delicate structure than that of *S. nodiflorum* or *S. nigrum*. The lamina consists of a single layer of palisade tissue with two to three layers of spongy parenchyma below. The epidermis is not so thickly cuticularised as in *S. nodiflorum*. As in the latter the leaf bears innumerable hairs, but these are longer and finer. Their walls although thickened are not so much so as in the other species described, nor are they decorated with the minute papillae. Each hair is divided into four to six cells which taper very gradually to the apex.

Most of the hairs are of the protective type, the apical cells being conical in form. A few of them are glandular, a rounded secretory head replacing the conical cell of the more usual type of hair. The cells of the stalk are similar in all respects in both healthy and infected plants to those of the protective hair.

The individual hair cell is much more elongated than in the previously described forms (Fig. 34). The wall is lined with cytoplasm and many fine strands stream across the central vacuole. As in *S. nodiflorum* the contents of the cell are continually moving. Strands of cytoplasm disappear from view, others appear in new places; they anastomose and may fuse together completely or they may break away again. The nucleus is embedded in plasm which carries it around the cell. Sometimes it is suspended in the vacuole by fine strands, at others it rests against the cell wall or it may be rolled along the wall by the flowing of the plasm. The plasm contains mitochondria and minute globules of oil. Except in the very young leaves, there are no plastids present in the hair cells.

In many respects the histology and the cytology of *S. nodiflorum*, *S. nigrum* and *S. lycopersicum* are very similar. The chief disparity lies in the greater delicacy of structure throughout the latter.

The diseased plant.

Inclusions similar in form to those of *S. nodiflorum* occur in the tomato when it is attacked by aucuba mosaic. As in other hosts the first symptoms of the disease are not visible until several days have elapsed after infection (23). At about the time of appearance of macroscopic symptoms the cytoplasm becomes more conspicuous, its streaming is accelerated and innumerable minute particles appear in it; by successive fusions these increase in size (Fig. 35). They are carried about the cell by the plasm. By aggregation and fusion large protein masses are built up (Figs. 36, 37, 38). As they become larger, their speed of movement about the cell decreases. In the course of a few days, practically all the particles are contained within a single mass. This becomes more or less rounded, but its contours are often not so smoothly curved as in *S. nodiflorum*. Its substance condenses and the body gradually becomes more compact (Fig. 39). In the hair cells it usually retains a granular appearance, but in other tissues it may become more homogeneous. Vacuolation may occur as in *S. nodiflorum* and *S. nigrum*. Its chemical nature is similar to that of the bodies produced in these two species.

As in other hosts a spike-like crystal appears (Fig. 41). Very occasionally a third type of inclusion body is found which also occurs in some of the other hosts examined, but only very rarely. It consists of large hexagonal crystalline plates or of irregular polygonal plates which appear to be built up from a number of hexagonal ones of varying sizes lying edge to edge (Fig. 40). In side view these are rectangular. On addition of weak acids they become striate. These inclusions appear to be identical with the striated bodies commonly associated with tobacco mosaic diseases (6). However, in this case they occur so abundantly in the diseased tissue as to be described as "characteristic products of the reaction of the cells to the presence of the mosaic virus"; whilst with aucuba mosaic if they are found at all it is only very occasionally and then only in isolated cells.

As in *S. nodiflorum* the inclusions persist for some weeks, the containing cell being practically at rest. Then the spherical inclusion body crystallises out (Fig. 41). After several months the inclusions begin to dissolve. Finally, all inclusions disappear and the cell is indistinguishable from one of an old but healthy plant.

Inclusions are formed almost as abundantly as in *S. nodiflorum* and are distributed very similarly, but usually they do not attain the same dimensions. Their diameter may reach about 25μ in the basal cells of the hairs, but usually they are considerably smaller. They are formed in most of the hair cells of an infected leaf irrespective of green and chlorotic areas. However, they are seldom found in the hairs on the stems and petioles. In the leaf epidermis and especially in the elongated cells covering the veins, patches of cells in both green and yellow parts may contain inclusion bodies as in *S. nodiflorum*. Again, bodies are found in the palisade tissue, but only very infrequently. When they do occur, several adjacent cells are always so affected. When, as occasionally happens, the leaves of the host assume the "fern-leaf" condition, intracellular inclusions are particularly well developed in the tegumentary tissues.

Nicotiana tabacum.

The normal plant.

The leaf of *N. tabacum* is more fleshy than those of the forms previously described. In transverse section it shows one or two layers of palisade tissue with about three layers of parenchymatous tissue below. It is enclosed by a thinly cuticularised epidermis, the surface of which is covered by hairs. Most of the hairs are glandular, each consisting of a stalk of about four elongated cells with a globular head made up of one to three cells which are filled with protein material and a bundle of crystals. The hairs may branch, each branch ending in such a globular secretory head. A few are not of the glandular type and terminate in a conical cell. The walls of the hair are comparatively thin.

The cells of the stalk vary in size, the basal ones often being relatively large. They are elongated, tapering gradually to the globular head. The wall is lined with streaming plasm in which are embedded mitochondria, oil globules and frequently plastids. There appears to be more cytoplasm in these cells than in those of the previously described forms. The cell contents are in a continuous state of motion. Strands of cytoplasm are often thrown across the vacuole, but usually anastomoses are frequent and part of the cell may be occupied by a meshwork of streaming plasm. The nucleus is embedded in the cytoplasm and with the other inclusions it is carried about the cell (Fig. 42).

The diseased plant.

As in the other forms described, infection with aucuba mosaic produces on the leaves of tobacco a characteristic irregular mottling. There is, however, no apparent difference in the thickness and development of the leaves in the green and yellow areas. A sharp histological difference of this nature has been described as occurring in mosaic disease of tobacco (6, 10). No such arrested development has been observed in any of the hosts infected with aucuba mosaic and so far described (cf. *Hyoscyamus niger*). Intracellular inclusions are produced regularly in tobacco on infection with aucuba mosaic. They occur in many of the stalk cells of the hairs; in the secretory cells at the apex they have not been observed, but their presence might be obscured by the normal contents of these cells. They are found in localised areas of the epidermis scattered over the leaf irrespective of chlorotic and "healthy" tissues, and occasionally in the palisade tissue. Here again this disease shows differences from tobacco mosaic, for with the latter inclusions are stated usually to be limited to chlorotic areas where they are abundant in hairs, epidermis and palisade (6).

The mode of formation of these inclusions in all tissues is similar to that just described for the three *Solanum* species. At the end of the incubation period, the streaming of the cytoplasm quickens, minute particles appear in it and aggregate to form large masses (Figs. 43, 44, 45). Most usually the body rounds off and behaves as in *S. nodiflorum* (Figs. 45, 46), but fairly frequently it remains as a large irregular mass of granular protein material. This may become vacuolate. Later, a spike crystal is often formed (Fig. 46). After some weeks the body crystallises out (Fig. 47). It is not known whether these crystals ultimately dissolve as, seven months after inoculation, they were still present in the cells. The chemical reactions of particles, inclusion body and the crystals to which it gives rise are similar to those already described for the inclusion bodies in other hosts infected with this disease. No other types of inclusion bodies were found in any of the tissues. No striated bodies were observed, but it cannot be said that these are never formed as they are seen only very rarely in any host infected with aucuba mosaic.

Hyoscyamus niger.

The normal plant.

In transverse section the healthy leaf shows a single layer of closely packed elongated palisade cells with innumerable chloroplasts. Below this are several layers of spongy tissue with well-marked intercellular spaces. The epidermis is only slightly cuticularised, the whole surface of the leaf lamina and petiole being covered with long hairs. The majority of these are glandular, the stalk consisting of from three to as many as eight or nine elongated cells and bearing a small globular secretory head of about three cells.

Each of the stalk cells is thin walled. The walls are lined with cytoplasm and the thin strands which flow across the vacuole often become enmeshed. Mitochondria and oil globules are carried about the cell by the flowing of the plasm, and plastids are embedded in the lining layer. The nucleus also is carried passively about the cell (Fig. 49).

The diseased plant.

Hyoscyamus is very badly affected by aucuba mosaic, seedlings often succumbing to the disease a few days after inoculation. The leaves show the characteristic mottling of aucuba mosaic, this symptom being unusually brilliant.

The cytological effects also are marked. At first the hair cells are affected in a similar way to those of the other hosts described. All movement within the cell is accelerated, then particles appear and circulate in the plasm (Fig. 50). They increase enormously in number and aggregate into large masses which lie apparently quiescent and usually against the cell wall. Occasionally these protein masses round off to give the typical form of inclusion body of aucuba mosaic in *S. nodiflorum* or *S. nigrum*, but usually there is no contraction of the mass and no rounding of its contours, the aggregations of particles being spread irregularly throughout much of the cell (Figs. 51, 52). They retain their granular form and may become vacuolate.

Often a spike crystal appears but no striated bodies. After a time the protein masses begin to crystallise out (Fig. 54). It is not known what would be the ultimate end of the protein masses and the crystals to which they give rise as the plants usually succumb completely to the disease. These inclusions are contained by all the hairs on the yellow areas, but seldom by those in the green tissues.

An attack of aucuba mosaic has a marked histological effect on the leaves of *H. niger* comparable to that of mosaic disease of tobacco. The chlorotic areas of the leaves are much thinner than are the healthy green areas of the plant. This is due to arrested development of the leaf tissues. The palisade cells are not elongated as in the normal leaf, and the intercellular spaces in the spongy tissue are poorly developed. The chloroplasts are destroyed or never develop. Irregular protein inclusions such as are formed in the hair cells are produced in practically every cell of the parenchymatous and epidermal tissues. No such inclusions are formed in the green areas of the leaf (Fig. 53).

DISCUSSION.

The nature of the inclusion bodies.

Much has been written concerning the nature of intracellular inclusions produced as a result of infection with virus diseases of both plants and animals. Frequently a disease may produce several differing types of inclusion body. Some of these are similar to inclusions occurring in healthy plants and are obviously reaction products of the cell; the striate material accompanying tobacco mosaic and the spike crystal of aucuba mosaic of tomato are of this order. Possibly many of the bodies described as being associated with a particular virus disease are not produced as a direct result of infection; Klebahn (11, 12) described spirally twisted thread-like bodies, "scolecosomes," as occurring in the phloem cells of *Anemone nemorosa* infected with "Alloiophyllie," but later identical inclusions were found in healthy anemones.

However, it is to the rounded protoplasm-like inclusion that so much significance has been attached. Various theories have been advanced as to the origin and significance of this body. The views as to its nature have been summarised by Rivers (20) and Ludford (15) for the diseases attacking animals and by Henderson Smith (24) for those attacking plants. In both cases the theories fall into three main groups: it has been suggested that they are parasitic organisms causing the disease; that they are products of interaction of the host cell and the virus or the result of cytoplasmic or nuclear reaction or degeneration; or that they consist of organisms combined with products of cellular reaction.

Protozoan organisms have often been described as occurring in association with virus diseases and have been presumed to be the causative agent. However, in a recent paper McLennan (17) describes a variety of *Leptomyxa reticulata* which is associated with a virus disease of hops. It occurs in some of the infected plants only and is regarded as a secondary invader; the resistance of the host having been lowered by the virus disease, the protomyxean form is able to enter the cells. Possibly other of the organisms described are such secondary invaders.

That the spherical bodies produced by aucuba mosaic are not organismal is shown by their mode of formation and their subsequent crystallisation and dissolution. It has been shown that they are formed entirely independently of the cell nucleus; their frequent association with it has already been explained. Indeed, with aucuba mosaic the nucleus of the host is entirely unaffected by the disease and in only one case in the plant kingdom, that of dahlia mosaic (7), has a definite invasion of the nucleus been shown. These bodies are apparently composed mainly of the products of cell reaction to the stimulus of the virus. Possibly they also contain the etiological agent of the disease, whatever its nature. The body is built up by the aggregation of particles of altered cytoplasm. Presumably the virus is dispersed throughout the cytoplasm, so that it is reasonable to suppose that it is contained within these particles.

However, the mode of formation of these bodies might account for much of the evidence which has led to the belief that they are living organisms. Many of the phenomena occurring in the living cell would be wrongly interpreted if observed only in fixed preparations. The fission figures that have been described and the presence of several bodies in one cell can easily be paralleled here (Figs. 21, 22, 38). After the rounding off there is no increase in size, rather there is a contraction, but in fixed preparations the variation in size from cell to cell might be regarded as evidence of growth of the body. Pseudopodia have been described also. The fusion of a larger and a small mass (Fig. 11) or an irregularly shaped body in process of formation (Fig. 15) or the breaking away of a portion of the body (Fig. 22) might easily be so interpreted in fixed preparations.

It seems probable that the intracellular inclusions of plants are of several different types as they are in animal diseases, and they may differ equally in their mode of origin. It is hoped that when more is known of them, of the reaction of many hosts to one virus and a comparison is made of many viruses affecting the same host, that they will be of use diagnostically. At present but little systematic cytological work of this kind has been carried out with any one virus. Very many diseases have been studied cytologically, but only on isolated host plants. It is known that tobacco mosaic produces similar inclusions in *Nicotiana tabacum*, *Capsicum annuum*, *Lycopersicum esculentum*, *Physalis pubescens*, *P. franchetti*, *Petunia violacea*, *Hyoscyamus niger*, *Nicandra physoloides*, *Solanum laciniatum*, *S. miniatum* and *S. atropurpureum* but in *Nicotiana glutinosa* and *N. glauca* inclusions are not formed (8). Cellular inclusions have not so far been found in plants infected with cucumber mosaic. Aucuba mosaic of tomato in the hosts examined always tends to produce bodies of a certain type. These, however, may not always reach the same degree of development; in *S. nodiflorum* etc., they are usually rounded, whilst in *H. niger* they remain as irregular masses. A study is at present in progress of these same hosts infected with tobacco mosaic, but until the work is further advanced it is preferred to draw no parallels between the two diseases. The type of inclusion formed appears to be specific to the virus, the formation of the body being the response of the host cell to some peculiar characteristic of the particular virus. However, in certain cases, the host may modify slightly the form of the body. In view of Hoggan's results it seems improbable that cytological characteristics will always serve as a means to identification of a particular disease, but when one host is susceptible to several diseases they should serve as a means of differentiation.

SUMMARY.

A description is given of the mode of formation of intracellular inclusions produced by aucuba mosaic of tomato in *Solanum nigrum*, *S. nodiflorum*, *S. lycopersicum*, *Nicotiana tabacum* and *Hyoscyamus niger*.

Soon after infection the rate of streaming of the cytoplasm is increased, then minute particles of protein appear in the cytoplasm which carries them passively about the cell. These particles aggregate and fuse to form large masses which are still carried passively but more slowly about the cell. These fuse until all the protein material is contained in one or occasionally more granular masses. In the three *Solanum* species examined this mass becomes rounded and it may lose its granular appearance and become vacuolated. In *N. tabacum* the body does not always round off and in *H. niger* it very seldom does so but remains as

an irregularly shaped granular mass which may, however, become vacuolate.

There is no evidence at any time of autonomous movement, the particles and the fully formed body being carried, as are the cell nucleus, mitochondria, etc., of the normal plant, in the cytoplasmic stream.

After the spherical body is formed a spike-like crystal appears in the cell.

The cell remains at rest for the space of several weeks. Often the rounded inclusion body and the nucleus are juxtaposed, but there is no special significance in this, it is merely the accidental result of the mode of formation of the body. Particles tend to accumulate where a number of strands of plasm meet; usually several strands converge on the nucleus.

Ultimately the body breaks down giving a number of protein crystals. After some months these dissolve. In *H. niger* the inclusion bodies are confined to the chlorotic areas where they are abundant in all tissues. In the other species studied they are distributed over green and yellow tissues. They are very abundant in the hairs, less so in the epidermis and very rare in the palisade and spongy tissues. In *H. niger* the development of the palisade tissue is arrested, in the other species the development is not so obviously affected although growth is retarded.

These inclusions appear not to be organismal in nature; they seem to be products of reaction of the host cell to the virus, but they may contain the etiological agent of the disease.

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Fig. 1 *a*.

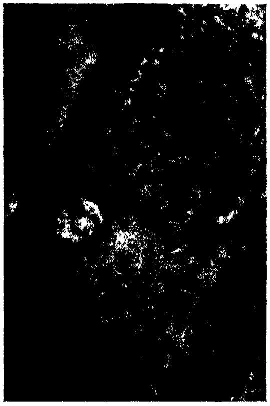


Fig. 1 *b*.

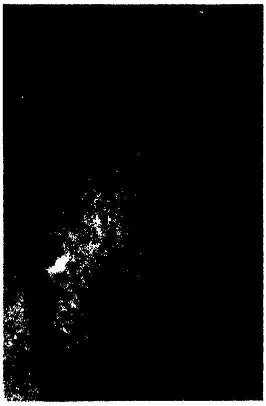


Fig. 2.

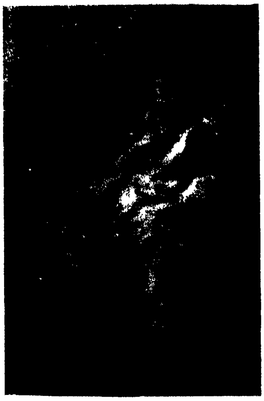


Fig. 3.

Fig. 4.

Fig. 5.

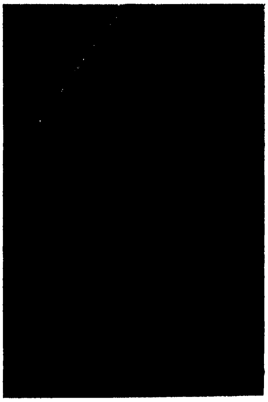


Fig. 6 *a*.

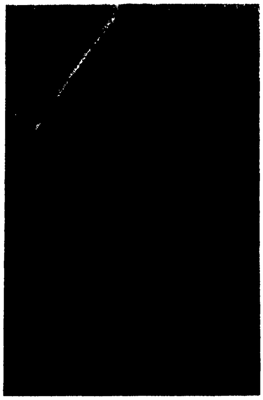


Fig. 6 *b*.

SHEFFIELD.—The Formation of Intracellular Inclusions in Solanaceous Hosts Infected with Aucuba Mosaic of Tomato (pp. 471–493).

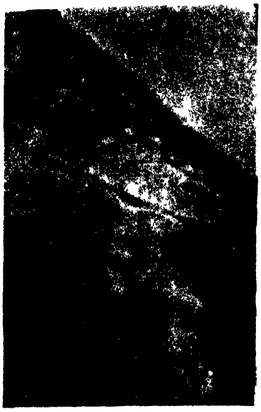


Fig. 7 *a*.

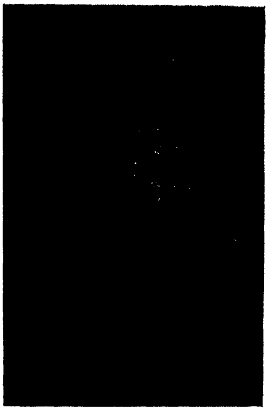


Fig. 7 *b*.

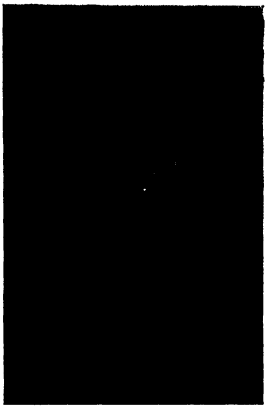


Fig. 7 *c*.

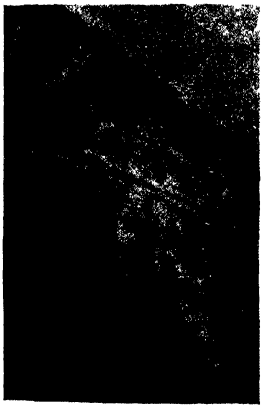


Fig. 7 *d*.

Fig. 7 *e*.

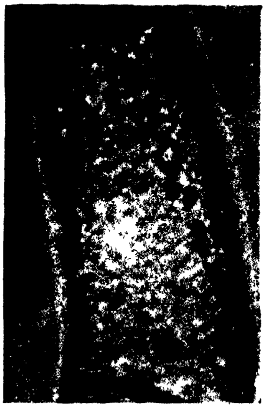


Fig. 8.

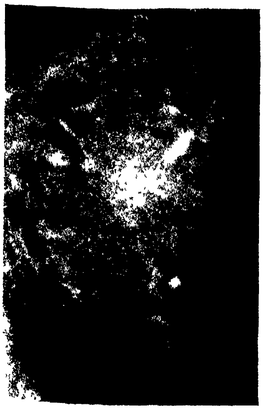


Fig. 9.

Fig. 10 *a*.

Fig. 10 *b*.

SHEFFIELD.—THE FORMATION OF INTRACELLULAR INCLUSIONS IN SOLANACEOUS HOSTS INFECTED WITH AUCUBA MOSAIC OF TOMATO (pp. 471–493).

EXPLANATION OF PLATES XXV—XXXIII

Figs. 1–20 are enlargements from single pictures of a cinematograph film demonstrating the effect of the disease on the cells of the hairs of *S. nodiflorum*. The film was taken using a Leitz No. 5 objective and a I ocular. The magnification on the negative was × 225. The prints were enlarged three times, but in reproduction they have been reduced by ⅓, giving a final magnification of × 450.

All other figures were taken with a Leitz Micca camera. A Leitz 6L objective combined usually with a Leitz × 10 periplanat eyepiece were employed giving a magnification on the negative of × 150 at a tube length of 170 mm. The prints were enlarged 2¼ times and have been reproduced without further alteration. Magnification × 340.

For Fig. 25 a Leitz periplanat ocular × 12 was used—this gave a magnification of × 180 on the negative. The print was enlarged 2¼ times increasing the magnification to × 400.

All figures are taken from unstained living cells.

A mottled appearance across the cell in many of the earlier figures is due to the papillose thickening of the cell wall.

PLATE XXV.

Solanum nodiflorum.

Fig. 1 *a*. Part of a cell from the hair of a healthy plant. The nucleus is near the wall. Several fine strands of cytoplasm are flowing more or less parallel to the long axis.

Fig. 1 *b*. The same cell 2½ minutes later. The nucleus has moved slightly out of focus. Most of the cytoplasm previously apparent has disappeared, and a very fine thread now runs obliquely across the vacuole.

Fig. 2. Part of hair cell of infected plant. Minute particles are visible in the cytoplasm, especially in that surrounding the nucleus.

Fig. 3. Larger particles are visible in the rather conspicuous cytoplasm. The nucleus is suspended in the vacuole by plasmic threads.

Fig. 4. Similar protein particles in the basal hair cell and in the epidermal cells.

Fig. 5. Numerous particles carried in the layer of plasm lining the wall and in strands crossing the vacuole.

Fig. 6 *a*. A similar stage to Figs. 3–5. Note two particles moving together along a strand of cytoplasm.

Fig. 6 *b*. 24 seconds later these two particles fuse.

PLATE XXVI.

S. nodiflorum, cont.

Fig. 7 *a–e*. Fusion of particles. Two particles are moving slowly along a thread of cytoplasm, a second pair approach rapidly behind them and all join together. 12 seconds elapse between *a* and *b*, 6 between *b* and *c*, 3 between *c* and *d*, and 3 between *d* and *e*.

Fig. 8. Numerous particles moving about the cell.

Fig. 9. Particles increase in size.

Fig. 10 *a* and *b*. A large plastic mass of protein material is drawn across the cell by a cytoplasmic strand, changing its shape as it travels. Other protein material is collected near the nucleus. 36 seconds elapse between *a* and *b*.

PLATE XXVII.

S. nodiflorum, cont.

Fig. 11. The nucleus is suspended in the vacuole by a number of strands of cytoplasm. Large aggregations of protein material are accumulating near it.

Fig. 12 *a*. As in Fig. 11, protein material is accumulating around the nucleus which is suspended in the vacuole. Large masses of this material are brought to the nucleus often only to be torn away again.

Fig. 12 *b*. However, 25 minutes later a considerable amount of fusion has taken place.

Fig. 13 *a*. The nucleus is flattened against the cell wall. Two masses of protein approach a third which is lying against the transverse septum.

Fig. 13 *b*. 2½ minutes later, these three masses have commenced to fuse.

Fig. 13 *c*. 2 minutes later, all the protein material has moved away from the transverse wall and is juxtaposed to the nucleus.

Fig. 13 *d*. After another 2 minutes, the fusion of the protein masses is apparently complete. (The nucleus has disappeared from view behind the developing inclusion body.)

Fig. 13 *e*. But 3 minutes later, the protein masses fall apart again.

PLATE XXVIII.

S. nodiflorum, cont.

Fig. 14 *a*. A mass of protein material is travelling along the cell wall towards two other masses which are slightly out of focus.

Fig. 14 *b*. 48 seconds later the first mass has changed its form in rolling along the wall and has passed slightly out of focus. The other two masses now begin to move towards it.

Fig. 14 *c*. 36 seconds later all three masses fuse just out of focus.

Fig. 15. The protein material is drawn into a single irregularly shaped mass.

Fig. 16. The mass becomes smoother in outline but retains its granular appearance.

Fig. 17. It becomes rounded and more homogeneous but vacuoles may appear in its substance. A few small particles still circulate in the cell.

Fig. 18. The completely developed inclusion body may lie apposed to the nucleus.

Fig. 19. The nucleus and a much vacuolated inclusion body are widely separated but each is resting against a cell wall. The cytoplasm still streams around the periphery of the cell and a single strand flows across the vacuole.

Fig. 20. A spike-like crystal appears in the cell.

PLATE XXIX.

S. nodiflorum, cont.

Fig. 21 *a*–*d*. Two spherical inclusion bodies have been formed in this cell. When apparently about to fuse they are drawn apart. Later they again approach each other.

Note also the movement of the developing body in the upper cell.

10 minutes elapse between *a* and *b*; 30 between *b* and *c* and 15 between *c* and *d*.

Fig. 22. Part of this inclusion body is about to break away.

Fig. 23. Vacuolate inclusion bodies.

Fig. 24. After some weeks the bodies break down into protein crystals.

Fig. 25. Six months after inoculation all inclusion bodies have disappeared from the cells.

PLATE XXX.

Solanum nigrum.

Fig. 26. The basal cell of the hair of a normal plant. The nucleus, containing nucleoli, is suspended in the vacuole by numerous cytoplasmic strands which radiate to the cell wall. Plastids are embedded in the parietal plasm.

Fig. 27. An early stage in the development of inclusion bodies in an infected plant. Innumerable small particles of protein material have been deposited on the surface of the nucleus.

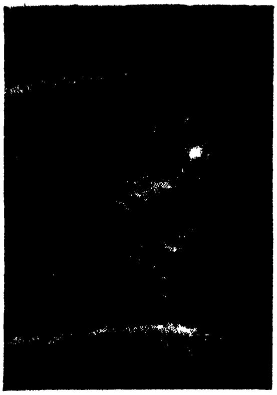


Fig. 11.

Fig. 12 *a*.

Fig. 12 *b*.

Fig. 13 *a*.

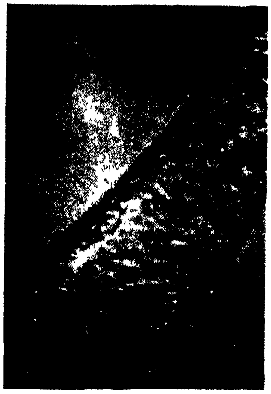


Fig. 13 *b*.

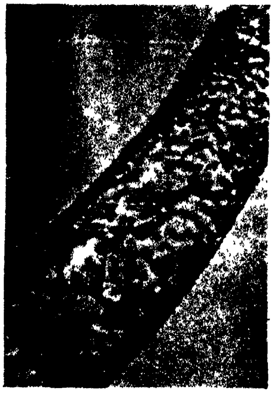


Fig. 13 *c*.

Fig. 13 *d*.

Fig. 13 *e*.

SHEFFIELD.—The Formation of Intracellular Inclusions in Solanaceous Hosts Infected with Aucuba Mosaic of Tomato (pp. 471–493).

Fig. 14 *a*.

Fig. 14 *b*.

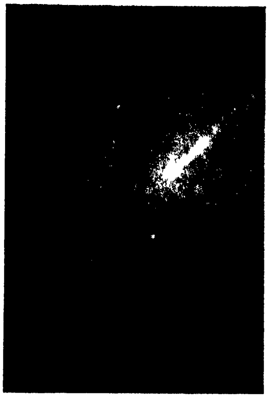


Fig. 14 *c*.

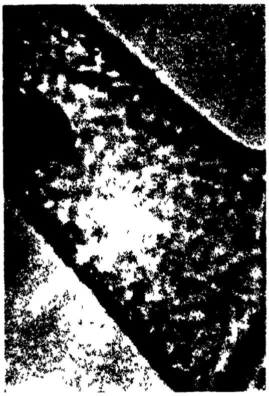


Fig. 15.

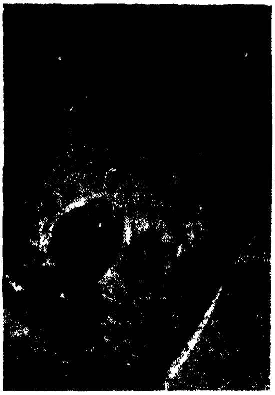
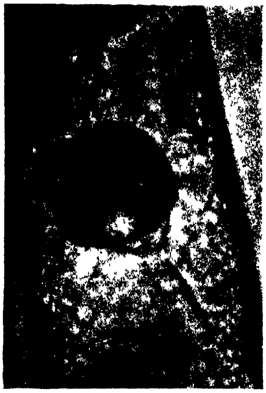


Fig. 16.



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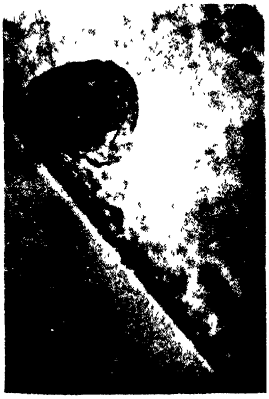

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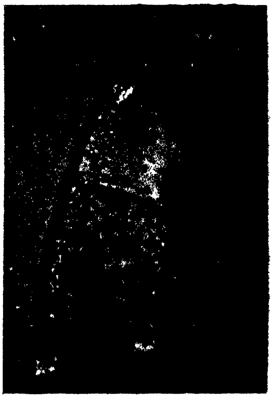

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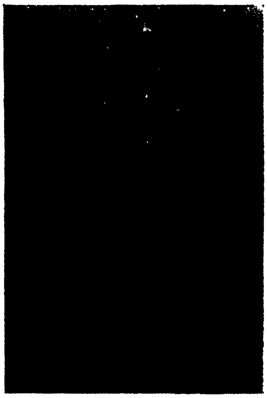


Fig. 20.

SHEFFIELD.—The Formation of Intracellular Inclusions in Solanaceous Hosts Infected with Aucuba Mosaic of Tomato (pp. 471–493).

Fig. 21 *a*.

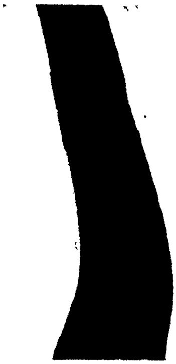


Fig. 21 *b*.

Fig. 21 *c*.

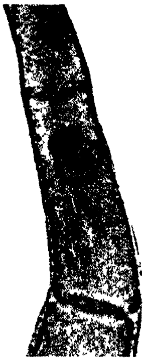


Fig. 21 *d*.

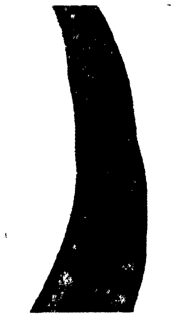
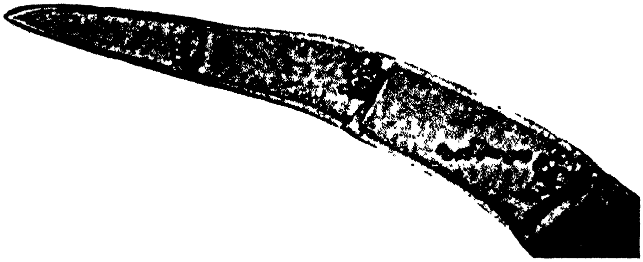


Fig. 22.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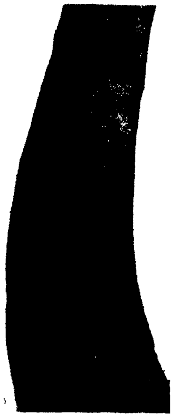
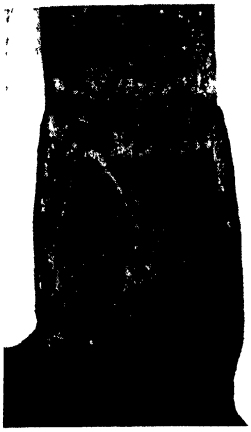


Fig. 23.

Fig. 25.

SHEFFIELD.—The Formation of Intracellular Inclusions in Solanaceous Hosts Infected with Aucuba Mosaic of Tomato (pp. 471–493).



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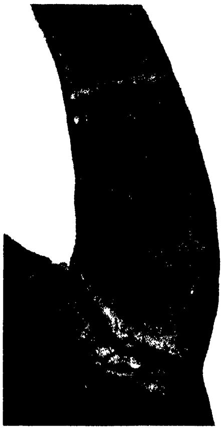


Fig 27.

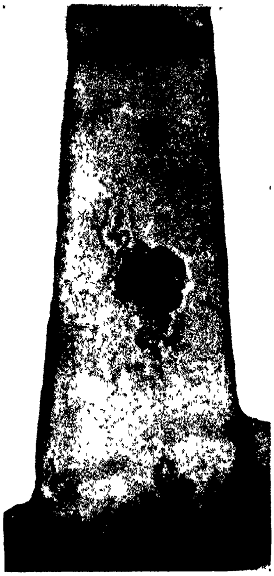


Fig 28

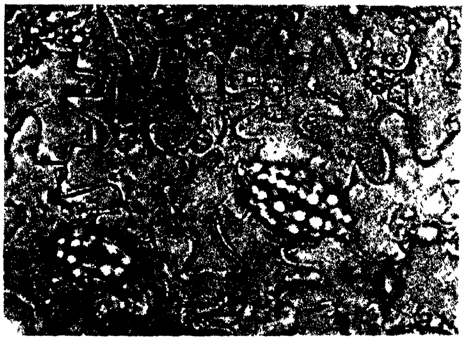
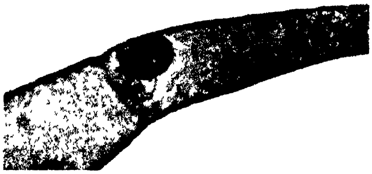


Fig 29.



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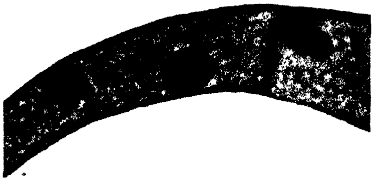
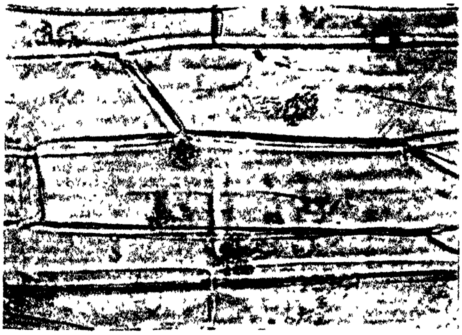


Fig 32



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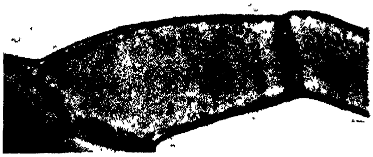


Fig. 33.

SHEFFIELD.—The Formation of Intracellular Inclusions in Solanaceous Hosts Infected with Aucuba Mosaic of Tomato (pp. 471–493).

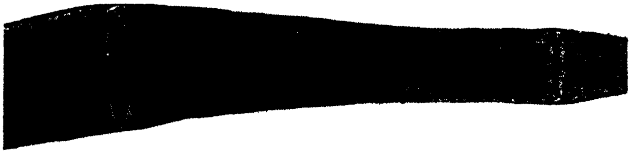
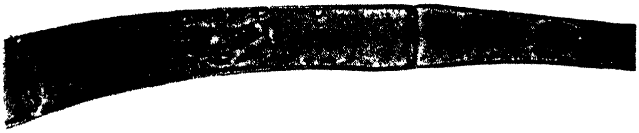


Fig. 34.



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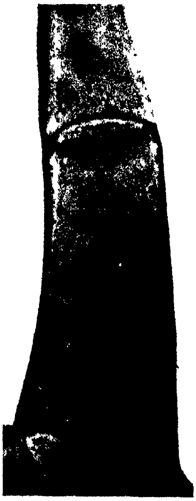


Fig. 35.

Fig. 37.

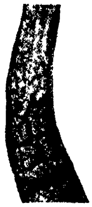


Fig. 40.

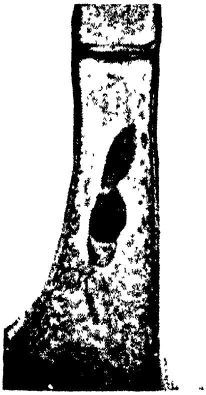


Fig. 38.

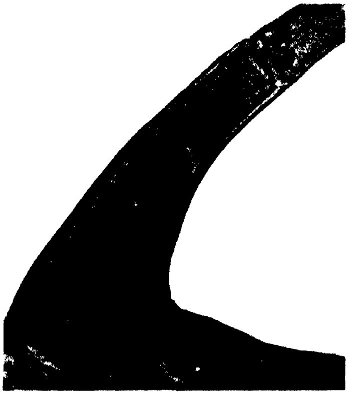


Fig. 39.

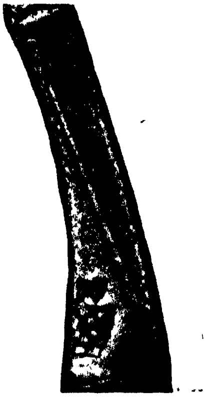


Fig. 41.

SHEFFIELD.—The Formation of Intracellular Inclusions in Solanaceous Hosts Infected with Aucuba Mosaic of Tomato (pp. 471–493).

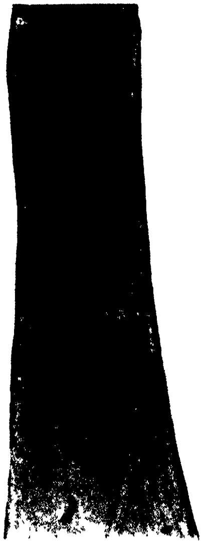


Fig. 42

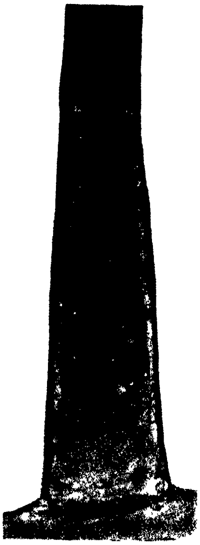


Fig. 43

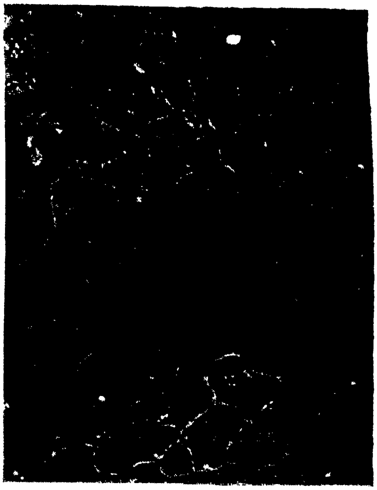
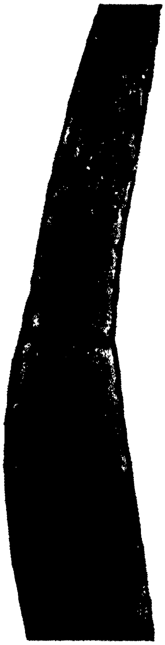


Fig. 48.

Fig. 44.



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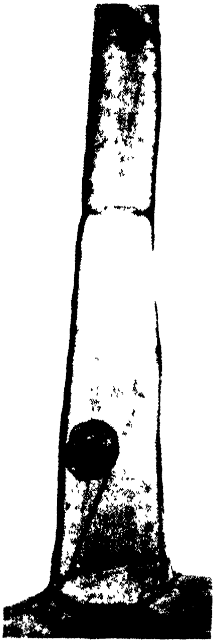

Fig. 46.

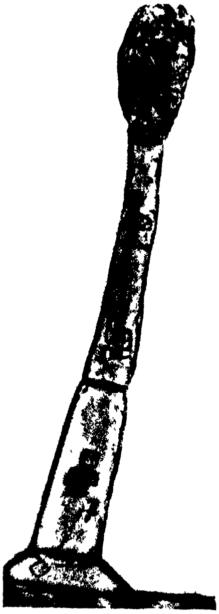

Fig. 47.

SHEFFIELD.—The Formation of Intracellular Inclusions in Solanaceous Hosts Infected with Aucuba Mosaic of Tomato (pp. 471–493).

Fig. 49.

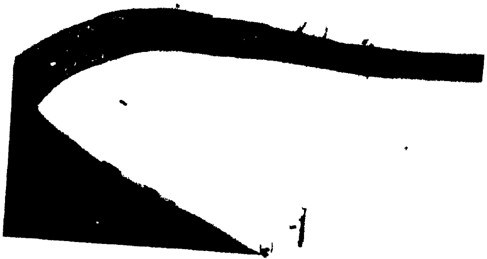

Fig. 51.

Fig. 50.

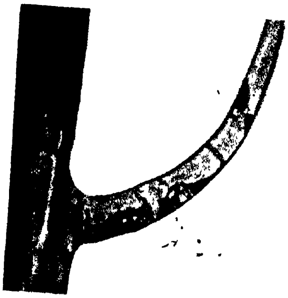

Fig. 52.

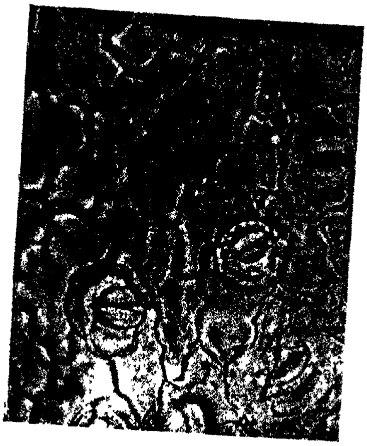

Fig. 53.

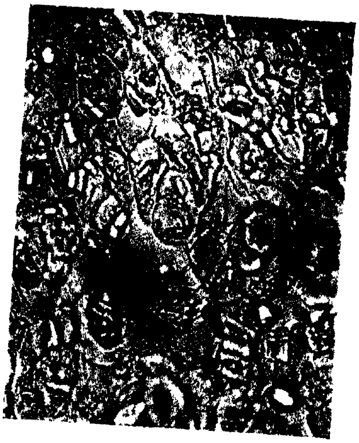

Fig. 54.

SHEFFIELD.—The Formation of Intracellular Inclusions in Solanaceous Hosts Infected with Aucuba Mosaic of Tomato (pp. 471–493).

Fig. 28. Large aggregations of protein material circulate in the cell.

Fig. 29. Developing inclusion bodies in the epidermal cells.

Fig. 30. Inclusion bodies developing in the epidermis from above a vascular bundle.

Fig. 31. A developing inclusion body is closely apposed to the nucleus.

Fig. 32. Fully formed inclusion bodies.

Fig. 33. After several months, the inclusion bodies have disappeared from the cells.

PLATE XXXI.

Solanum lycopersicum.

Fig. 34. A cell from the hair of a healthy plant. The nucleus is suspended in the vacuole by fine strands of cytoplasm.

Fig. 35. Soon after infection small protein particles circulate in the cytoplasmic strands. They have already formed one aggregation which is approaching the nucleus.

Fig. 36. Several aggregations circulate in the cell and fuse. .

Fig. 37. Protein masses moving about the cell.

Fig. 38. The particles have all been drawn into two masses one of which rests against the nucleus.

Fig. 39. A fully formed inclusion body lying against the nucleus.

Fig. 40. Striate material is occasionally formed.

Fig. 41. The spherical body has crystallised but the spike crystal is still present.

PLATE XXXII.

Nicotiana tabacum.

Fig. 42. Part of a hair cell of a normal plant. The nucleus lies against the thin cell wall. Numerous plasmic threads cross the vacuole. Plastids are embedded in the parietal plasm.

Fig. 43. Soon after infection small aggregations of protein particles move about the cell. A number of these are collected near the nucleus.

Fig. 44. Larger protein masses move about the cell. The majority are collecting together near the nucleus.

Fig. 45. In the upper cell most of the protein material is now contained in one large irregular mass. A fully formed body lies apposed to nucleus and wall in the lower cell.

Fig. 46. The cell contains a granular, vacuolate inclusion body, a spike like crystal and the nucleus.

Fig. 47. A small glandular hair. Inclusion bodies which were formed in the stalk cells, have now crystallised.

Fig. 48. The bodies formed in the epidermis have crystallised.

PLATE XXXIII.

Hyoscyamus niger.

Fig. 49. Cells from the apex of a glandular hair of a normal plant. The stalk cells each contain streaming cytoplasm, a nucleus and plastids.

Fig. 50. Soon after infection. A few minute particles have appeared in the cytoplasm.

Figs. 51 and 52. Large aggregations of protein material lie against the cell walls.

Fig. 53. The epidermis from the green area of the leaf is exactly like that of a normal leaf. The cells contain cytoplasm and nucleus but no inclusion bodies.

Fig. 54. In the chlorotic areas of the leaf, the epidermal cells are smaller. They contain large masses of protein material which crystallise out.

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STUDIES IN THE PHYSIOLOGY OF VIRUS DISEASES IN PLANTS

III. AUCUBA OR YELLOW MOSAIC OF TOMATO IN *NICOTIANA GLUTINOSA* AND OTHER HOSTS

By JOHN CALDWELL, B.Sc., Ph.D.

(*Department of Mycology, Rothamsted Experimental Station, Harpenden.*)

(With Plate V.)

The purpose of the present paper is to put on record some experiments with aucuba or yellow mosaic of tomato which have been carried out on solanaceous hosts and to direct attention especially to the use of *Nicotiana glutinosa* with this virus, in experiments of a more general nature.

A detailed description of the symptoms induced by aucuba mosaic in tomato (*Lycopersicum esculentum* var. Kondine Red) has already been given (1). In tomato, *Nicotiana rustica*, *Solanum nodiflorum*, and *S. nigrum*, this virus induces mottling in the leaves and, occasionally, in the fruit. Similar symptoms appear in tobacco (*N. tabacum* var. "White Burley") but this host is exceptional in that it also develops local symptoms at the point of inoculation. In all these plants intracellular inclusions have been found. Further, the disease is systemic and the symptoms are not confined to the region of inoculation.

The chlorosis which is the typical symptom in these host plants is apparently due to an inhibition of the formation of chlorophyll, rather than to an actual destruction of the preformed chloroplasts. This is clearly indicated by the observation that chlorosis affects the leaves round the growing point, *i.e.* those leaves which have developed *after* the inoculation of the plant.

Inoculation is effected by rubbing the leaves of the experimental plants with macerated tissue of infected tomato leaves. No local lesions appear as a consequence of this treatment except in tobacco, which is remarkable in its reaction to this disease. In the plants under discussion, there is little alteration in the starch content of the cells of leaves which were mature at the time of infection (cf. Holmes (2)). When, however, leaves which had developed or which were developing symptoms of mosaic are treated with alcohol to remove the chlorophyll and then with iodine, the absence of starch from considerable areas can readily be demonstrated. There is apparently little starch in the regions which are chlorotic, and very often the vascular bundles are included in those regions. In older leaves, which do not show symptoms, the starch is uniformly distributed over the tissues.

In another group of solanaceous plants, however, the symptoms induced by inoculation with aucuba mosaic juice are quite different since the primary symptoms appear on the rubbed leaf. The rubbing of the leaf breaks the hairs and through the broken hairs the virus agent enters the tissues. Within five days in the Rothamsted Experimental Station glasshouses necrotic spots appear on the treated leaves. They are generally distributed over the leaf-surface and are associated with the broken hairs of the adaxial side. These spots are about 5 mm. in diameter, are roughly circular in outline and increase only slightly in size for a few days. The tissues are shrunken and dry and the leaves are consequently distorted and crinkled by the collapse of the cells. No other symptoms are visible on the treated plants and no systemic infection occurs. Reactions of this type are found in *Datura stramonium*, *N. acuminata*, and *N. glutinosa* of the species which have been examined.

N. tabacum occupies an intermediate position between the first and second groups, in that local lesions appear on the rubbed leaves and, later, mosaic symptoms appear systemically over the whole plant. In the second group no intracellular inclusions are formed in the cells of the affected leaves and the virus is not generally recoverable from tissues of the host plant other than those of the leaves inoculated in the first instance. No movement of the virus takes place across a leaf of *N. glutinosa* of which only a portion has been rubbed with virus juice.

THE MOVEMENT OF THE AGENT ACROSS THE LAMINA.

A series of leaves of *N. glutinosa* were rubbed, some on the distal portion of the lamina, others on the proximal portion and others on one side of the midrib. In no instance was any virus recovered from the unrubbed portion of the leaves. When minimal doses were rubbed on leaves and, after symptoms had developed, these leaves were macerated with water and inoculations were made back into tomato the majority of the plants did not become infected. Since tomato is so easily infected, this suggests that the amount of virus present in the *Nicotiana* was small.

Plate V, fig. 1 shows the appearance of a plant of *N. glutinosa* inoculated by rubbing virus on the four lower leaves. The photograph was taken a fortnight after treatment and it can be seen that the rubbed leaves developed well-marked symptoms of the disease and that the rest of the plant was normal.

It was noticed that in our glasshouse occasional old plants of *N. glutinosa* developed mottled leaves at the top. This mottle occurred on some of the plants with rubbed leaves, and systemic secondary infection was suspected. Inoculations were made from this material into tomato and on the leaves of other plants of *N. glutinosa.* No symptoms developed on these plants so that, despite the superficial resemblance, the mottle was not causally associated with the aucuba mosaic.

That this species may develop systemic infection with other viruses has been clearly shown. When inoculations were made by rubbing inocula of "experimental streak" on the lower leaves local lesions appeared within a few days and systemic infection occurred later. The systemic infection was a fine mottle induced by the potato mosaic virus which was a component of the "experimental streak," the latter being a mixture of the virus of aucuba mosaic and of potato mosaic. The mixed viruses induce systemic mosaic and necrotic symptoms in the tomato. The virus of aucuba mosaic in *N. glutinosa* therefore induces similar symptoms to those found by Holmes for the virus of tobacco mosaic. Holmes has suggested that the number of spots on the rubbed leaves of *N. glutinosa* and *N. rustica* is an index of the concentration of the virus agent in the infective material (3).

The "drying out" of the areas on the leaves of *N. glutinosa* resembles closely the "drying out" which occurs not infrequently on the leaves of tomato plants infected with severe aucuba mosaic. The characteristic feature of this "spotting" is that the tissues are brown rather than black, since little melanin pigment is formed in the dead areas as occurs, for instance, in "streak" disease. The tissues are desiccated and their appearance suggests a severe local wilt. Similar symptoms may appear in tomato plants infected with aucuba mosaic when kept in the glasshouse, and do appear if similarly infected tomato plants are kept in the dark for some days.

The fact that leaves of *N. glutinosa* react to aucuba mosaic as they do to tobacco mosaic indicated that they might well be used for the demonstration of the presence of this virus in a juice. It had previously been noted that the virus of aucuba mosaic multiplies and induces symptoms in young detached tomato leaves kept with their petioles in damp sand. It seemed reasonable to suppose, therefore, that if *N. glutinosa* leaves were detached from the plant, inoculated by rubbing and left in Petri dishes or on damp sand, symptoms would develop after the usual period of inoculation.

For the purpose of the experiment infected tomato leaf tissue was macerated with distilled water in the proportion of 1 gm. of leaf tissue to 2 c.c. of water. This material was passed through a fluted filter paper impregnated with fuller's earth. The filtrate was dark brown in colour and contained no chloroplasts. Inoculum prepared in this way was considered as the standard inoculum in all the experiments recorded in this paper and all dilutions were made on that basis.

THE DETECTION OF THE PRESENCE OF THE VIRUS AGENT IN JUICE.

Filtered virus juice was diluted to 1/10 and two drops of this material were rubbed on the surface of detached leaves of *N. glutinosa*, which were kept with their petioles in damp sand under a bell-jar in the glass-house. Within 48 hours the first sign of "spotting" appeared on the leaves. Control leaves which had been rubbed with water or with healthy juice were normal. The rubbing was done with the index finger, care being taken to rub the inoculum evenly over the surface without damaging the rather soft tissues of the mesophyll. On the third day, that is within 72 hours, the rubbed areas were dry and brown. The appearance of the leaves is shown in Plate V, fig. 2. Excellent results have been obtained by rubbing the surface of the leaves with juice diluted to 1/100 and thereafter laying the leaves on damp filter paper on the bottom of Petri dishes. These were left on the laboratory table and definite symptoms appeared within 48 hours. This technique is valuable in that it affords a quick and reliable method of determining the presence of the agent, at least of aucuba mosaic, in a suspected juice. This method has the further advantage of approximating even more closely to the methods of culturing bacteria on plants than does that of Holmes (3), from which it is adapted.

Little importance can be attached to the conditions under which the detached leaves are maintained, but they must always be kept moist either in the light or in the dark. There is some evidence, however, that if they are kept under too wet conditions the appearance of symptoms may be delayed or partially suppressed. This suggests that wilting is the main factor in the development of symptoms. The number of spots varies considerably with various factors (cf. Holmes) but the number is always large enough to demonstrate the presence of the virus. Mere mechanical injury does not cause the "spotting." The area of the groups of dead cells does not increase in size after the first few days but remains more or less unchanged after they have become delimited. The cells of the leaves do not contain the intracellular inclusion bodies often found in association with the disease, nor do the other cells of the plant present any abnormal appearance. There is no evidence of much, if any, multiplication of the virus in the affected leaves.

This latter point is difficult of exact assessment in that there is always a certain amount of virus juice adherent to the rubbed surface. On the other hand, when the inoculum was used in low dilution, *e.g.* 1/10, in no experiment did more than a few of the experimental plants secondarily inoculated develop symptoms. After rubbing, the leaves of the *Nicotiana glutinosa* were washed in a stream of water to remove all the surplus inoculum which adhered to the surface. The material was inoculated into groups of eight tomato plants and, at most, two or three in each group developed symptoms, while sometimes no plants of the group showed any trace of symptoms. When the *N. glutinosa* leaves were treated with alcohol to remove the chlorophyll and were put into an aqueous solution of iodine in potassium iodide, starch was found to be present in the cells of the lamina except in the region immediately round the lesions. There was a small "halo" of tissue without stain round each lesion but no spread of the clear tissue down the neighbouring vascular tissue as found in tobacco leaves by Holmes.

The appearance of the spots can readily be seen in the photograph on Plate V, fig. 2. On the leaf photographed the area of dead tissue is clearly delimited and the damage is purely local.

Two hypotheses suggest themselves in the interpretation of these phenomena. Either (*a*) the effect of this virus, which in so many of the Solanaceae causes definite mottling, is completely changed in this particular plant by some substance or condition which is not found in the others, or (*b*) the immediate effect on the tissues of this plant is so violent that a region of tissue round the point of inoculation is completely killed so that no virus is able to enter living cells of the mesophyll (cf. Caldwell (4)).

THE MOVEMENT OF THE VIRUS AGENT INTO UNBROKEN CELLS.

Various experiments have indicated that the virus agent is unable to enter unbroken cells. It has been found that infection does not follow wetting the surface of the leaf of tomatoes etc. with aucuba mosaic juice, nor does it occur when juice is forced directly into the xylem vessels (4). It appears, therefore, that the epidermis of the leaf and the walls of the vessels and of the living cells round them act as barriers to the agent. Similarly, the root-hair seems to be impervious to the agent while the wall is intact, since it is possible to grow seedling tomato plants on cotton wool soaked in virus juice and, though the roots are bathed in the juice, the plants grow fairly rapidly and remain free from infection. Again, watering plants with virus juice in no case has given rise to symptoms, though infection can take place through a broken root. If, however, inoculations be made from the roots of diseased plants there is every indication that the virus content of these roots is considerable.

The observations that infection does not follow wetting with virus the unbroken cells of the epidermis of a plant or of the vascular tissue or of the roots, support the view that the agent is unable to enter an unbroken cell of a plant. The objection could, however, be raised that the epidermis is covered with a cuticle which is largely water-proof, and that the junction between the xylem vessels and the living parenchyma is obviously not of a simple membrane type. The same objection does not appear to hold for the root-hair but, admittedly, the root-hairs are a rather specialised mechanism.

One type of cell seemed to be particularly suitable for experiments of this type and that is the mesophyll cell. Especially did the leaves of *N. glutinosa* appear to furnish useful material in that detached leaves of this plant show symptoms within 48 hours. It was assumed that these symptoms could and did appear only when rupture of the cells had taken place (cf. tomato experiments).

THE INJECTION OF THE LEAVES OF *N. GLUTINOSA* WITH VIRUS JUICE.

For these experiments the virus juice after filtration was diluted to 1/10 and was put into a glass vessel of suitable size. The leaves to be treated were removed from the plants so that no hairs on the laminae were broken, and the leaf was invariably held by the petiole. Through the end of the petiole at right angles to the lamina was pushed a long pin, by which the leaf could be suspended into the virus juice and the lamina completely submerged. The juice had no access to the damaged region of the petiole and the pin was so placed on the top of the jar that the lamina did not knock roughly against the sides. The vessel was put under a bell-jar attached to a suction pump and the air in this jar was then exhausted to a considerable extent whereby the gas in the intercellular spaces of the leaf was removed. When the tension was released the juice entered by the stomata and filled the intercellular spaces. The leaves under experiment weighed some 2–3 gm. and took up about 2 c.c. of juice, approximately their own weight of juice. The appearance of the leaf was naturally altered by this treatment, the leaf being darker in colour and almost translucent by transmitted light. The leaves were set out to evaporate most of the superfluous juice from the intercellular spaces and were then fixed by the pins to frames under the bell jars which kept the air moisture-laden. Alternatively, the pins were removed and the leaves were laid on moist filter papers or cotton wool spread on the bottoms of Petri dishes. The removal of some, at least, of the juice in the intercellular spaces was especially necessary in the case of those leaves which were afterwards kept in Petri dishes, since the complete injection of the leaves apparently induced anaerobic respiration and this, in turn, led to the destruction of the tissues of the mesophyll. This consideration did not arise to the same extent in the less well-saturated atmosphere under the bell jars. In none of these leaves did any spots appear after the preliminary experiment. In this experiment five leaves were used and on each of three of these a single necrotic spot appeared. In the later experiments the leaves were invariably normal after treatment.

The controls were of two groups. Some leaves were treated in a manner similar to that described, with the exception that they were rubbed on the surface after treatment. "Spots" appeared on these leaves. Other leaves were rubbed with virus juice on the surface and, in these cases also, symptoms invariably appeared. The amount of virus rubbed over each leaf was approximately 0·1 c.c. and in these experiments at least 15 spots appeared on each leaf treated. The difference in the amounts of juice in the uninfected leaves and the infected rubbed control leaves was considerable. Nevertheless, in the leaves with the unbroken cells the larger quantity of juice was apparently unable to cause infection since there is no means of entry into the mesophyll cells.

THE MOVEMENT OF THE VIRUS IN PLANTS WHICH DO NOT DEVELOP SYSTEMIC SYMPTOMS.

Reference has been made to the fact that in some of the Solanaceae the agent of aucuba mosaic is apparently not found in quantity in regions other than those directly inoculated. An examination was made of the possibility of movement of the agent through tissues in which it was not producing symptoms. For this purpose an experiment was set up in which tomato shoots were grafted as scions on to stocks of *Datura stramonium* and *vice versa.* Four groups of plants were set up for the experiment, tomato plants, *Datura* plants, *Datura*/tomato grafts, and tomato/*Datura* grafts. Two leaves on each of the plants were rubbed with aucuba mosaic juice. In the first group of the grafts two leaves of the tomato portion were rubbed and in the second two leaves of the *Datura.*

In the ungrafted tomato plants no symptoms appeared on the treated leaves but systemic mosaic symptoms appeared, as usual, after ten days. In the ungrafted *Datura* plants necrotic lesions appeared in the rubbed leaves and, very occasionally, on the stem above a rubbed leaf. This latter phenomenon is not unusual, though no explanation suggests itself as to why the isolated necrotic areas should develop sporadically over the plant. Henderson Smith (5) has found that these are the regions from which the virus agent is recoverable on subsequent inoculation.

When the grafts were examined it was found that necrotic areas had developed on the rubbed leaves of the *Datura* after four or five days, and that mosaic symptoms subsequently appeared in the tomato whether scion or stock. When, however, the virus juice had been rubbed on the leaves of the tomato portion mosaic symptoms never appeared on the *Datura*.

In *Datura* therefore the presence of the agent passing through the plant is apparently not sufficient to induce the formation of the necrotic areas which are characteristic of the disease but that some other factor is involved in the expression of disease symptoms.

SUMMARY.

It has been shown that the symptoms induced by aucuba or yellow mosaic of tomato in certain other members of the Solanaceae (notably *N. glutinosa* and *D. stramonium*) differ markedly from those in tomato. Neither formation of intracellular inclusions nor systemic infection occurs in these plants. In *N. glutinosa*, the symptoms appear only on the rubbed portion of the leaves and little multiplication of the virus takes place. In *D. stramonium*, although no mosaic symptoms appear on the host, the virus travels through the tissues and can infect susceptible grafts. Holmes' work on the use of *N. glutinosa* as a ready means of demonstrating the presence of the virus agent in a juice has been confirmed and amplified.

It has also been shown that it is possible to inject the intercellular spaces of the leaf of *N. glutinosa* with virus juice and that no infection occurs unless cells have been ruptured.

This work was carried out under the auspices of the Empire Marketing Board.

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EXPLANATION OF PLATE V.

Fig. 1. Plant of *N. glutinosa* with symptoms of aucuba or yellow mosaic of tomato on the lower leaves.

Fig. 2. Leaf of *N. glutinosa* with symptoms of aucuba or yellow mosaic of tomato.

(*Received October* 10*th*, 1931.)

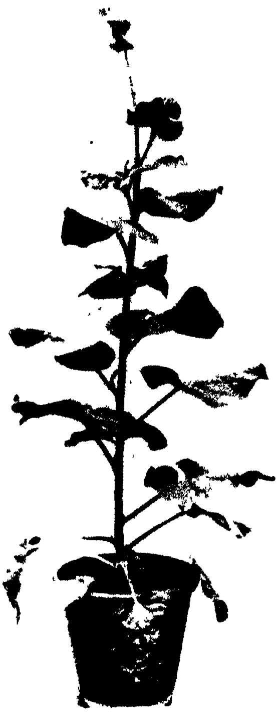

Fig 1

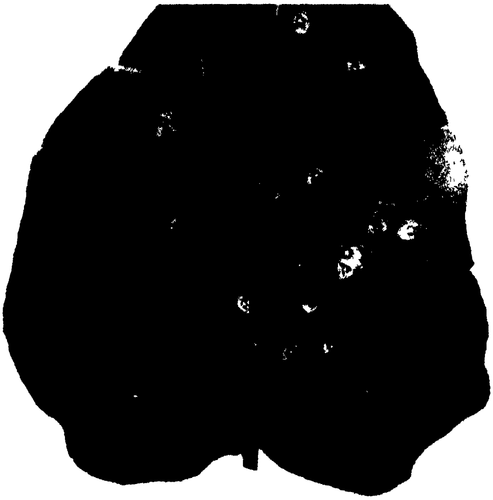

Fig 2.

CALDWELL.—STUDIES IN THE PHYSIOLOGY OF VIRUS DISEASES IN PLANTS (pp. 144–152).

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ON THREE NEW VIRUS DISEASES OF *HYOSCYAMUS NIGER*

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(With Plates XXVIII—XXX.)

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INTRODUCTION.

This paper describes a group of virus diseases infecting commercially grown *Hyoscyamus niger* (Henbane). The observations were made in the fields of Messrs William Ransom and Son, Ltd., Manufacturing Chemists of Hitchin, Bedfordshire, to whom I am indebted for facilities in the field and for material for experiments.

Hyoscyamus is grown for the leaves and the flowers which are used for the manufacture of pharmaceutical preparations. The plant is grown as a biennial crop sown, as a rule, in October, though in a bad autumn it may be held over until February. In the first year of growth two leaf crops are taken in July or early August, and late September. In the second year a flower crop is harvested in June but two or three rows are generally kept for seed until August. After the flower crop is taken the roots are ploughed in and a rotation crop, wheat, potatoes, sanfoin or sugar beet, is grown. Messrs. Ransom grow the *Hyoscyamus* in three large plots in such an order that there is always one plot in leaf, one plot in flower and one plot under the rotation crop. Two of the plots are in one large field and the other in a field about a mile away. During the year in which the plots were under observation, July 1930 to September 1931, which involved two growing seasons of the *Hyoscyamus*, the rotation crop was sugar beet.

METHODS.

All the plants used in the experiments, unless stated to be taken direct from the field, were grown from known virus-free seed under as nearly as possible insect-proof conditions. Direct inoculations were made by the needle method (scratching through a drop of fluid from macerated leaves) or by rubbing with cotton wool. The insect cultures were kept in a specially designed insectary in cellophane cages (1). Stock insects were reared on cabbage or spinach which do not take or carry the diseases. The infected stock cages were enclosed, for greater precaution, in cupboards of phospho-bronze gauze, and the cages were only opened in the small culture chamber, which adjoins the insectary but is completely sealed off from it. The culture chamber was thoroughly sprayed with either pyrethrin or nicotine solutions after each experiment.

The insects were transferred on sterile camel-hair brushes, each insect being taken from the infected plant on to filter paper and transferred with a second brush, to prevent the virus being carried by touch. Infected insects were placed in suitable numbers on batches of young seedlings, which were kept as long as the insects were alive in lamp-glass cages in the insectary until after spraying, when they were removed to heated insect-proof glasshouse chambers.

PRELIMINARY OBSERVATIONS.

For convenience the first field containing the two plots will be referred to as Field A, plots 1 and 2, and the second as Field B. The first disease was obtained from Field A, plot 1, in September 1930 during an investigation of a flea-beetle attack. It was noticed in this plot that some of the plants were stunted and necrosed sometimes with deformation of the leaves and "rosetting" of the habit. The crop was at the end of its first year just before harvesting. The diseased condition was at first attributed to the effects of the beetle attack but, when a closer examination revealed mottling of the leaves and yellowing or "clearing" of the veins, experiments were devised to determine whether a virus disease were present.

Experiment I. Winter buds from the crown of the tap roots were grown in Rothamsted plots free from the beetle; some of these plants showed stunting and mottling as in the field and some were normal. Plate XXVIII, fig. 1, shows three plants of which two are diseased and one is normal. Juice from the stunted and normal plants were needle-inoculated into young healthy seedlings and those inoculated from the stunted plants showed symptoms of disease.

Experiment II. Leaves from infected plants in the field were macerated and inoculated into healthy seedlings grown at Rothamsted; these also produced definite symptoms.

In the spring of 1921 an attempt was made to obtain more infected plants from Field A, plot 1, but the field had been ploughed ready for the rotation crop and plot 2 was only newly planted. Specimens were therefore taken from Field B, the crop of which was already in its second year. Juice from these plants and from plants from Field A, inoculated into *Hyoscyamus* produced different symptoms. The two fields continued to give these distinct characteristic symptoms whenever specimens were taken throughout the thirteen months during which they were under observation. Later experiments showed that there were two groups of diseases. The first group, from Field A, plots 1 and 2, was found to consist of two components, a "vein band" and a "yellow mosaic" type which were called Hy. II and Hy. III respectively with a possible third which has been temporarily called "Green *Hyoscyamus* mosaic" (4). The second group (from Field B) contained apparently only one virus of the "ring spot" type which is known as Hy. IV.

VIRUS DISEASES FROM FIELD A.

Direct inoculation.

Hy. I.

Hy. I was the name arbitrarily given to the disease, as it came from the field before any information was obtained as to whether it was caused by one or more viruses. Juice from the presumed infected leaves was inoculated by needle into a batch of young *Hyoscyamus* seedlings as already mentioned. The resulting symptoms took the form of dark green spots on the older leaves. A second series of inoculations was made from field plants into large batches of *Hyoscyamus* and tomato seedlings.

All the *Hyoscyamus* seedlings showed the same symptoms, but the tomatoes were different in their reactions. Six out of eight inoculated tomato seedlings showed no symptoms, but two produced symptoms of extreme stunting with dark coloration, blistering and deformation of the leaves (Plate XXVIII, fig. 2). When inoculated back into tomato, *Hyoscyamus* and tobacco, juice from these stunted plants gave the same result in tomato. In *Hyoscyamus* and tobacco it caused a violent "yellow mosaic" with a tendency to broad dark green bands along the veins, necrosis of the older leaves, and some deaths among the younger or weaker plants (Plate XXVIII, figs. 1 and 2).

The first suggestion arising from these observations was that Hy. I was a complex of two viruses, one producing mild "green" symptoms and one violent "yellow" symptoms. In order to distinguish these they were given numbers; Hy. II for the mild and Hy. III for the violent disease. It was supposed, despite the discrepant fact that the Hy. III only showed in tomato though it is also capable of completely masking Hy. II in *Hyoscyamus*, that they were associated in the field and would continually occur together in inoculation. This, however, did not happen. The disease (Hy. III) obtained from the original tomatoes remained throughout a long series of inoculations involving about 600 plants of various kinds, constant to the distinct type of symptom and to other properties, physical and physiological, which will be described later, but on no other occasion was recovered from the field. Also it did not appear in any plants inoculated from the original Hy. I in *Hyoscyamus* or from the subsequent inoculation with Hy. II.

Later observations on Hy. II showed that it apparently never produces symptoms in tomato. In *Hyoscyamus* and tobacco it produces "vein band" symptoms in the young leaves (Plate XXIX, figs. 3 and 4) which persist throughout life in tobacco but not in *Hyoscyamus*. In this plant they disappear and become, in the older leaves, a dark green irregular mottle such as is described for Hy. I. Subsequent inoculation from infected field plants gave rise to "vein band" symptoms in the same way, and it is possible that they also appeared in the original Hy. I inoculation but were not observed as they disappear rapidly in *Hyoscyamus*.

Hy. III.

Host range. The detailed description of Hy. III is given first as it is more characteristic in its symptoms and properties than Hy. II, and therefore more easy to deal with. Also the symptoms are more definite and the percentage of infection is higher than with Hy. II. The percentage of infection by scratching (needle method) or by rubbing is very high and cases of failure to "take" are very rare unless the juice has been kept for some time or passed through fine filters. In addition to tomato, *Hyoscyamus* and tobacco it causes disease in the following hosts:

Nicotiana glutinosa—a systemic infection of vein band symptoms similar to those caused by Hy. II in tobacco, but tending to become fainter in the older leaves. A striking characteristic of the disease in *N. glutinosa* is the "breaking" of the flower which is normally self-coloured pink, but in plants infected with Hy. III is white striped with pink. The symptoms take longer to show in *N. glutinosa* than in any other host—about fourteen or fifteen days as compared with five to seven days.

Nicotiana glauca—violent necrosis with blistering of leaves and stunting.

Petunia sp.—yellowing of regions round veins with necrosis of the older leaves.

Datura stramonium—symptoms similar to those in *Hyoscyamus*—yellow mosaic with tendency to dark green vein bands and blistering.

Hy. III causes no disease in the following Solanaceae: *Solanum nodiflorum*, *S. dulcamara*, *S. melongena* (Egg plant), *Atropa belladonna*, *S. tuberosum* (Potato)—the following varieties: Arran Chief, Arran Victory, President, Epicure, Great Scot; or in the Cruciferae which were tested as food plants for aphids: *Reseda odorata* (Mignonette), *Raphanus raphanastrum* (Radish), *Brassica oleracea* (Cabbage), *B. rapa* (Turnip). Two garden plants were also tested with negative results, viz. *Myosotis sylvaticum* and *Ageratum officinalis*.

Symptoms. In type the symptoms of Hy. III disease resemble those of the common tomato and tobacco mosaic diseases—aucuba (2), tobacco mosaic, and yellow mosaic of tobacco (Johnson, 6). The symptoms of Hy. III in tomato slightly resemble those of tobacco mosaic, but are more violent and the stunting is much more severe. Hy. III tomatoes present a characteristic "parsley head" appearance, in which the leaves are distorted into indefinite curly masses. (More detailed photographs of symptoms will be published in a later paper.) In tobacco the mosaic produced by Hy. III virus is more definitely yellow than tobacco mosaic but resembles the "yellows" disease. The type of symptoms shown in Plate XXIX, fig. 2, with the broad blistered dark green bands is characteristic of the younger plant. Later the bands become smaller and often necrotic at the edges, and the leaf becomes a confused chequered design of dark bands, yellow mottle and necrotic spots. In the old tobacco plant the young leaves often "grow out" of the symptoms and appear to be normal though the virus can be recovered from them apparently in full strength.

In *Hyoscyamus* the symptoms are almost always of the type shown in Plate XXIX, fig. 1, and do not vary much with age. In all three hosts the older leaves tend to become necrotic and die away at the base of the plant.

In *Hyoscyamus* and tobacco there are well-marked preliminary symptoms which take the form of a "yellowing" or "clearing" of the veins (Plate XXVIII, fig. 3). They appear on the first two or three leaves which show infection after inoculation. A similar appearance is sometimes detectable in very young tomatoes at the same stage of infection. Preliminary symptoms occur in needle, rubbed and aphid-transmitted infection.

In some cases, particularly with *Hyoscyamus* and tobacco, death is caused by the virus. It generally occurs in the early stages of the disease and is much more common in the spring than in summer and autumn. Out of 49 positive results in Hyoscyamus fairly evenly distributed over 8 months, March to October 1931, there were 14 deaths, 11 of which occurred from inoculations made between March and June. Deaths of tomato have not been so common, but this may be due to the fact that most of the tomato inoculations were made later in the season of 1931, as there is one outstanding case for this year (1932) in which 35 out of a batch of 40 inoculated in April died in about a fortnight. Deaths of *Hyoscyamus* and tobacco during the spring of 1932 have so far been about as common as in the previous spring, but no comparative figures can yet be given.

Filterability. Experiments were carried out on the filterability of Hy. III. They were made using juice from tobacco, tomato and *Hyoscyamus* on different occasions, in the proportion of 1 gm. leaf material to 3 c.c. distilled water. Inoculation was made by needle into batches of tomato, *Hyoscyamus* and tobacco plants both immediately after filtration and after being kept for 24 hours.

The filtration was made through, first, a filter consisting of alternating layers of sand and paper pulp followed by Chamberland filter candles L 1 and L 3. The results were as Table I.

It is thus apparent that Hy. III will not pass an L 3 candle and hence appears to have larger particles than most of the known plant viruses, or else to have some special property by which it is absorbed on to the porcelain.

Table I.

Filtration of Hy. III.

Plants inoculated	Preliminary filter		L 1		L 3	
	1 hour	24 hours	1 hour	24 hours	1 hour	24 hours
6 *Hyoscyamus*	+	–	+	–	–	–
6 tomatoes	+	–	+	–	–	–
6 tobaccos	+	–	+	–	–	–

Survival of the clarified juice. It appeared from the above tests that Hy. III juice will not keep in a clarified state for 24 hours.,Tests were also made to find out when it became uninfective. A series of tobacco plants were inoculated in batches of five at hourly periods with juice from tobacco filtered through an L 1 candle. After 24 hours two batches were inoculated with (*a*) the L 1 filtered juice, (*b*) unfiltered juice which had been kept as a leaf pulp.

Table II.

Survival of clarified juice 1931.

Plants inoculated	Period of keeping (hours)	Unfiltered juice (as leaf pulp)		L 1 filtered juice	
		Positive	Negative	Positive	Negative
5 tobacco	1	—	—	5	0
8 ,,	2	—	—	5	0
5 ,,	3	—	—	4	1
5 ,,	4	—	—	3	2
5 ,,	5	—	—	3	2
5 ,,	6	—	—	2	3
5 ,,	24	5	0	0	5

Similar results were obtained by inoculating batches of tomatoes at 2-hour periods for 8 hours. The suggestion from this experiment was that the clarified juice may last more than 8 hours, though it had lost a certain degree of its infective capacity in this time, and less than 24 hours. Unfortunately, as it was late in the season there were not large enough numbers of plants to repeat a long time experiment, but two further 24-hour experiments, using pulp filtered and L 1 juice, were made in September, both giving negative results.

These experiments were repeated in the spring of 1932 and the results did not agree with those of the previous autumn. Three experiments involving large numbers of plants were carried out, and in all cases the virus was infective after 24 hours. There seemed to be a slight diminution of virulence which can be observed in Table III.

Table III.

Results on April 10*th of inoculation from March* 23*rd,* 1932.
Clarified juice from tobacco.

Juice kept **Individual tomato plant**	**1 hr.**	**2 hr.**	**3 hr.**	**4 hr.**	**5 hr.**	**6 hr.**	**7 hr.**	**8 hr.**
1	×	0	×	×	×	√	√	√
2	0	×	0	×	×	0	0	√
3	×	×	×	0	0	0	0	√
4	0	0	0	0	×	0	×	0
5	0	×	0	0	0	0	×	0

× **dead plants.**
0 **dying plants (these all died during the week following the above observations).**
√ **ordinary symptoms.**

This experiment also demonstrates the lethal nature of the virus in the spring as compared with 55 inoculations made between August and October in the previous years in which no plants died—cf. p. 566. It has also been noted in three similar experiments that the longer the juice is kept the longer the period which elapses between inoculation and the appearance of the first symptoms. With juice kept for 24-hour periods the following results were obtained.

Table IV.

Survival of clarified juice 1932. *Juice from tomato.*

Juice kept 6 *Hyoscyamus*	1 hr.	24 hr.	48 hr.	72 hr.
1	+	+	0	0
2	+		0	0
3	+	+	0	0
4	+	+	0	0
5	+		0	0
6	+	+	0	0

It is seen that there is a definite disparity in the survival periods of clarified juice between the autumn of 1931 and the early spring of 1932.

Effect of heating. Specimens of juice from infected tomato and *Hyoscyamus* were heated for 10 minutes in thin-walled tubes in a water bath kept constant at various temperatures. They were then inoculated into batches of plants with the following results:

Juice from tomato.

50° C.	5 *Hyoscyamus*	5 pos.
60° C.	5 ,,	5 neg.
70° C.	5 ,,	5 ,,
80° C.	5 ,,	5 ,,

Similar results were obtained with juice from *Hyoscyamus* into tomato. Therefore Hy. III will survive a temperature of 50° C. but not of 60° C. or over.

Intracellular inclusions. Hy. III forms cell-inclusions in all three hosts; they are similar to aucuba and tobacco "X-bodies" but more loosely formed. In *Hyoscyamus* they are shapeless and often accompanied by spindle-shaped crystals. They have been seen in the hairs and epidermis (particularly along the veins of *Hyoscyamus* and in the hairs of tomato and tobacco).

Hy. II.

Host range. Hy. II has not been as extensively tested for hosts as Hy. III, but is similar in its reactions as far as has been ascertained except that it causes no symptoms in tomato. In *Nicotiana glutinosa* it gives, in the young plant, faint "vein band" symptoms which rapidly disappear. The same range of potato varieties has been tested both by needle inoculation and aphid transmission with negative results. This is important because in its symptoms Hy. II resembles K. M. Smith's Crinkle Y [(5) (Plate XXX fig. 1)]. Crinkle, according to K. M. Smith, is composed of at least two viruses which occur together in nature: Crinkle X, a non-insect transmissible virus which causes ring-spot symptoms, and Crinkle Y which has a "vein band" type of symptoms and is aphid transmitted. In all the potato inoculations control half-tubers were kept, into which true Crinkle (Myatt's Ash Leaf) was inoculated and gave positive results. Healthy tubers (except Great Scot) and infected Crinkle stock were obtained by the kindness of Dr Kenneth M. Smith and Dr R. N. Salaman from the Potato Virus Research Station, Cambridge.

Symptoms. As has already been stated, the symptoms of Hy. II are primarily of the "vein band" type both in *Hyoscyamus* and in tobacco. In *Hyoscyamus* the "vein band" is a dark puckered area along the vein with very little interveinal mottle and no yellowing (Plate XXIX, fig. 3). In tobacco the symptoms are a typical "vein band" such as is described by K. M. Smith [(5), (Plate XXX, fig. 1)] and are quite indistinguishable from the symptoms of Crinkle Y in tobacco. Crinkle Y, however, causes a fine necrotic mottle in tomato, whereas Hy. II causes no symptoms. According to K. M. Smith (6), *Hyoscyamus* crinkle causes "clearing of the vein within 7 days followed by characteristic darkening along the vein" but the photograph given on his Fig. 15, Plate 33, does not look like Hy. II in *Hyoscyamus*.

In *Hyoscyamus* the "vein bands" disappear on the older leaves, and in the older plants on the young leaves which come up looking quite normal. The older leaves, however, always show a mottle though it may be very faint. In tobacco the vein bands generally persist throughout the life of the plant. The symptoms of Hy. II take longer to appear than those of Hy. III, generally 10 days to a fortnight, and preliminary symptoms of the Hy. III type (clearing of the veins) are only occasionally faintly discernible. The percentage of infection is not so regular as in Hy. III and varies between 70 and 100 per cent., thus larger batches of plants have to be used for each experiment.

Filterability. Parallel experiments on filterability to those on Hy. III were carried out on Hy. II and the results were practically the same except that it was rare to get full infection from the L 1 juice. It therefore agrees with Hy. III in being unable to pass an L 3 filter.

Effect of heating. Similar results were obtained as with Hy. III except that no trials were made at 50° C. The virus became inactive after immersion for 10 minutes at 60° C. and over.

Intracellular inclusions. The tissues of leaves, stems and hairs were examined for intracellular inclusions, but no definite abnormal condition could be found. In some cases the cytoplasm of the epidermis in the elongated cells above the veins seemed to be rather irregularly thickened and darker than in the normal condition.

Insect transmission.

Hy. III.

The first insect tested as a vector of these *Hyoscyamus* diseases was the flea beetle found occurring with them (*psylliodes hyoscyami* Linn.). The flea-beetles were cultured in the insectary and fed on infected *Hyoscyamus*; after a week's feeding they were removed to a series of young *Hyoscyamus* and tobacco seedlings, none of which showed any sign of disease after a suitable period had elapsed. This experiment was repeated several times and always with the same result.

As the diseases in some ways resemble potato Crinkle, the next insect tested was the well-known vector of potato Crinkle and leaf-roll, *Myzus persicae* Koch. The same routine was followed, but this time the disease was successfully transmitted in nearly every case.

Subsequent experiments show that it is freely transmissible by this insect vector between *Hyoscyamus* and tobacco and *Nicotiana glutinosa*, but cannot be taken to or from tomato except by direct inoculation. The preliminary and secondary symptoms caused by aphid transmission are precisely the same as the results of direct inoculation. Lethal characters are equally common and show about the same periodicity.

Incubation period. The experiments to find the length of time for which the aphid vector remains uninfective after feeding on the infected plant are, like the survival of the clarified juice experiments, divided into two sets, the first performed in late summer and autumn of 1931 and the second in the spring of 1932. Also, like the survival experiments, they show a definite disparity in the results between the two seasons. Unfortunately at the end of 1931 it was not possible, owing to lack of material, to obtain conclusive results, Table V being the average of only two experiments on longer periods (of 24 hours and over) and one on shorter periods. It must be pointed out that other experiments done as controls at the same time with 24 hours on the infected and five days on the uninfected plants gave 100 per cent. infection, so that it is certain that the virus, aphids and plants were in a normal condition as regards general possibilities. Six presumed infected aphids were placed on each seedling.

Table V.

Experiments to test the period of time during which the vector of Hy. III remains uninfective.

Hyoscyamus plants	1st feeding on infected plant (hours)	2nd feeding on healthy seedling (hours)	Total hours	Result	
				Positive	Negative
5	2	16	18	0	5
5	4	16	20	0	5
5	2	22	24	0	5
5	4	22	26	0	5
4	6	24	30	0	4
5	2	34	36	0	5
5	4	34	40	0	5
5	6	48	54	0	5
4	4	60	64	2	2
5	6	72	78	2	3
5	6	96	102	5	0

The results seemed to indicate that there was a definite "incubation" period of about 3 days, possibly more to obtain full infection. However, when the experiments were repeated three times in March and April of 1932, using larger batches and more varieties of plants (*Hyoscyamus*, tobacco and *N. glutinosa*, using 6 to 12 plants for each period tested) it was found that infection could be obtained in 30 hours, 6 hours feeding and 24 hours on the seedling. Both *Hyoscyamus* and tobacco were used as sources of infection and, in two experiments of 30 hours from start to finish, gave 100 per cent. infection. The third gave a lower percentage infection throughout periods of 30, 54 and 78 hours and was possibly due to faulty feeding on the infected leaves.

Hy. II.

The percentage of transference of Hy. II by *M. persicae* is even lower than the percentage from needle inoculation. In some experiments it is very high but this cannot be guaranteed and the conditions regulating it are unknown. Infection is carried between *Hyoscyamus*, tobacco and *N. glutinosa*. The symptoms are the same as those caused by needle inoculation or rubbing. Tomato is apparently immune to aphid infection as it is to needle inoculation.

Incubation period. Only one experiment has so far been done on the incubation period of Hy. II. It was made in March to April of 1932.

Table VI.

Experiments to test the period of time during which the vector of Hy. II remains uninfective.

Plants	1st feeding infected *Hyoscyamus* (hours)	2nd feeding healthy seedlings	Total hours	Result	
				Positive	Negative
6 *Hyoscyamus*	6	24	30	0	6
6 tobacco	6	24	30	0	6
6 *Hyoscyamus*	6	48	54	1	5
6 tobacco	6	48	54	0	6
6 *Hyoscyamus*	6	72	78	3	3
6 tobacco	6	72	78	4	2
6 *Hyoscyamus*	6	96	102	2	4
6 tobacco	6	96	102	3	3

This indicates that 30 hours is definitely insufficient; 2 days' feeding gives one positive result which may or may not be significant, and 3 and 4 days give about the same percentage. The suggestion is that there is an uninfective period in the insect of about 2 days, but more experiments will have to be made to verify this. Nothing is known as yet as to whether there is any seasonal difference.

VIRUS DISEASES FROM FIELD B.

Hy. IV.

Host range. Hy. IV was obtained from Field B in May 1931. It was first thought to be the same disease as was affecting plants in Field A, but the first inoculation into *Hyoscyamus* proved this to be wrong. It has been obtained on several occasions from Field B and varies only in the strength of symptoms obtained at the first inoculation. Generally, after a few inoculations, they become identical, except in one case in which a strain brought from the field gave noticeably weaker symptoms even after passage through tomato. It gives 100 per cent. infection in *Hyoscyamus* and tomato. After passage through tomato it also gives positive results in tobacco. It has failed to cause any disease in the five varieties of potato, Arran Chief, Arran Victory, President, Epicure and Great Scot, which is one of its chief distinctions from Crinkle X which it otherwise resembles in symptoms (cf. K. M. Smith (5, 6)). Its infectivity throughout a much larger range of hosts is under examination.

Symptoms. In *Hyoscyamus* Hy. IV gives an all-over "pepper-and-salt" mottle slower in its appearance than Hy. III and not so vivid (Plate XXX, fig. 1). In some leaves this had a tendency to form rings, but these were never necrotic. The first few attempts to give the disease to tobacco failed altogether. In tomato it gave an "all-over" mottle similar to potato mosaic in tomato but, rather more of a "vein band" type (Plate XXIX, fig. 3). After passage through tomato it gave definite ring necrosis in *Hyoscyamus* (Plate XXIX, fig. 4) which, in the older plants, gave rise to a yellow mosaic, and also gave necrotic symptoms in tobacco (Plate XXIX, fig. 2). Similar symptoms in tobacco, though more of a mottle type with only occasional necrosis, were obtained without passage through tomato by inoculation from the original *Hyoscyamus* after about 3 months' growth. It is noteworthy that Crinkle X causes large necrotic lesions on the innoculated leaf [K. M. Smith (6) (Plate 33, fig. 16)] followed by ring-like lesions on the other leaves—whereas in Hy. IV the lesions formed by the virus after passage through tomato take the form of concentric rings on the inoculated leaves followed by ordinary mottle on the other leaves. It is not lethal in *Hyoscyamus* as is Crinkle X.

Hy. IV in tobacco is interesting in that symptoms appear first on the leaf next in order of growth to the one inoculated, and proceed in this order over the plant. In this way the older leaves become infective before the younger leaves which is directly opposite to the arrangement in many plant viruses in which the young leaves are the first to show infection. An experiment was arranged to determine whether the older leaves actually became infective before the younger ones:

A. A batch of six tobacco plants inoculated each on one leaf—(the oldest) from Hy. IV after passage through tomato. Symptoms did not show in *A* until the 11th day and then they appeared on the leaves (of the untouched pair) corresponding to those from which *B* and *C* were taken.

B. Four tobacco plants inoculated from the next oldest leaf of (two *A* plants) fourth day. Result—4 negative.

B′. Four tobacco plants inoculated from youngest leaf (two *A* plants)—fourth day. Result—4 negative.

C. Four tobacco plants inoculated from oldest leaf (two *A* plants)—ninth day. Result—2 positive, 2 negative.

C′. Four tobacco plants inoculated from youngest leaf (two *A* plants)—ninth day. Result—4 negative.

Filterability. Hy. IV can be filtered through an L 3 candle without any apparent diminution of infectivity.

Survival of clarified juice. The clarified juice of Hy. IV from tomato and *Hyoscyamus* is apparently unaffected by keeping for 48 hours and gives 100 per cent. infection.

Effects of heating. Heating in a water bath for 10 minutes at temperatures of 60°, 70° and 80° C. gave the following results.

Table VII.

Effect of heating Hy. IV.

Plants inoculated	60° C.	70° C.	80° C.
5 *Hyoscyamus*			
1	+	–	–
2	+	–	–
3	+	–	–
4	+	+	–
5	+	+	–

Similar results were obtained with tomato except that only one infection was obtained at 70° C. In one case an infection was obtained at 80° C. Hy. IV apparently survives 60° C. but its infectivity is impaired at 70° C. and almost entirely disappears at 80° C.

Intracellular inclusions. So far no traces of "X-bodies" or abnormal condition of the cells have been found in plants infected with Hy. IV.

Insect transmission. It has been found impossible to transmit Hy. IV by insects. *Myzus persicae*, *Macrosiphum gei* and *Thrips tabaci* have been tested as possible vectors without success. In this character it agrees with K. M. Smith's Crinkle X.

DISCUSSION.

It is not proposed in this paper to draw any definite conclusions from the results so far obtained, but there are some problems which arise out of this work which are of interest. These may, for convenience, be tabulated as follows:

(1) The relationship between Hy. II and Hy. III.

(2) The relationship between Hy. II and Hy. IV as compared with Crinkle X and Y.

(3) The inability of *Myzus persicae* to transmit Hy. III to or from tomatoes.

(4) The disparity in results between experiments with Hy. III in autumn 1931 and spring 1932 and the possibility of a seasonal variation in the virus.

(1) It is fairly obvious that the relationship between Hy. II and Hy. III is very close as they both originated from the same inoculation and are very similar in their reaction. Both are non-filterable through an L 3 candle, both are killed at 60° C. and both are carried by *Myzus persicae* into the same range of hosts. The main problem is that Hy. III only appeared on one occasion and then only in tomato though, if it were present in the inoculum, it should also have given symptoms in *Hyoscyamus* as it is capable of completely masking symptoms of Hy. II. It is possible that Hy. II does exist in Hy. III plants and cannot be seen. This suggestion is supported by one experiment[1] in which Hy. III was inoculated from tobacco into *Hyoscyamus* and gave, in two cases, normal Hy. III symptoms and in one only symptoms of Hy. II. The fact that Hy. II causes no symptoms in tomato and has not yet been recovered from it, does not prove that it could not be present in the presence of Hy. III. This, however, does not explain the sudden appearance of Hy. III for which there would seem to be only three possible explanations.

(*a*) That it is an accidental infection which occurred after the first inoculation into *Hyoscyamus* and tomatoes and affected two of the latter with a virus which resembles the original inoculum exactly except for the violence of the symptoms, which is quite unique in some of its properties and which has never occurred before or since in the Rothamsted glasshouses. This is very unlikely.

(*b*) That it occurred in the one plant of Field A which happened to be used for this inoculation and in no other which was used previously or subsequently, and that, by some accident, it failed to produce symptoms in the *Hyoscyamus* and produced them only in two of the tomatoes.

(*c*) That it is a variant, mutant or dissociate from Hy. II induced by the sudden change in the conditions in which the virus was reared (from the field to hot-house condition) and the change in host plant.

The number of coincidences demanded by explanations (*a*) and (*b*) render these highly improbable and in view of recent researches on variation and dissociation of bacteria and lower fungi, explanation (*c*) would seem to be the most probable. It is possible also that Hy. III itself is not simple and that another principle can be separated out by filtration (4) but this is still in process of investigation.

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[1] This result has occurred again since going to press.

(2) Although Hy. II and Hy. IV do not form a natural group like Hy. II and Hy. III, the similarity between them and the group of diseases which forms potato Crinkle is very striking.

Table VII.

Comparison between Hy. II, Crinkle Y, Hy. IV and Crinkle X.

Hy. II	Crinkle Y	Hy. IV	Crinkle X
Vein band symptoms in *Hyoscyamus* and tobacco, followed by mottle in *Hyoscyamus*	Vein band symptoms in tobacco. Clearing of veins followed by vein-band symptoms in *Hyoscyamus*	Ring necrosis in *Hyoscyamus* and tobacco after passage through tomato: followed by mottle	Ring spot symptom in tobacco. Necrotic lesions followed by rings in *Hyoscyamus*
Carried by *M. persicae*	Carried by *M. persicae*	No insect vector	No insect vector
Will *not* go by needle or aphid into: Tomato, Arran Victory, Arran Chief, President, Epicure	Will go by needle and aphid into: Tomato, Arran Victory, Arran Chief, President, Epicure	Will *not* go by needle into: Arran Victory, Arran Chief, President, Epicure	Will go by needle into Arran Victory, Arran Chief, President, Epicure
Not filterable through L 3	L 3 filtration (no record)	Filterable through L 3	Filterable through L 3
Not found with Hy. IV in nature	Found in nature with Crinkle X and alone in Epicure	Not found in nature with Hy. II	Found in nature with Crinkle Y

Table VII is a comparison of various characters of Hy. II and Hy. IV, as investigated so far, with similar characters of Crinkle X and Y as described by K. M. Smith (5, 6). It can be seen that though they are very much alike in some of the symptoms, in insect transmission, in having the same host plant, and possibly in filterability, there are sufficient important differences to mark them as separate entities.

One of the important differences is their occurrence individually in separate fields so close together, as it is unlikely that diseases which originated in potato and afterwards infected the *Hyoscyamus* (or *vice versa*) should do so in such a way that they occur together in one crop and separately in the other.

At present the most likely hypothesis is that they constitute two parallel series of viruses occurring in separate hosts, of which the members of each set are not related to each other but to their parallels in the other series. Possibly the relationships are something in the nature of that between Hy. II and Hy. III which has a host difference in tomato.

(3) The fact that the disease (Hy. III) is not transmissible by *Myzus persicae* to or from tomato is interesting in view of the results obtained by Hoggan (3) on tobacco mosaic. In her experiments, tobacco mosaic is transmissible by *Myzus pseudosolani* from tomato to tobacco, but not from tobacco to tobacco or tomato. The suggested explanation is that "the aphis does not extract the virus from the tissues of the tobacco plant on which it feeds" (3). This, however, does not cover a case in which an insect which can be proved to be infective fails to transmit the disease, *i.e.* an aphid infected from tobacco and feeding on tomato. No explanation as yet presents itself to account for these facts.

(4) The discrepancy between results of experiments made in the autumn and the spring occurs in three aspects of the investigation. Data regarding the lethal symptoms come from spring and autumn of 1931 and spring 1932. The other two, namely the survival of the clarified juice and the existence or not of a so-called "incubation period" of the virus in the insect, only apply to the autumn of 1931 and the spring of 1932. It is obvious that further data are needed, at least results from August to October 1932, before any definite postulation can be made. The appearance of lethal characters in the spring suggests either a seasonal alteration of the virus or a seasonal increased susceptibility on the part of the plants. This might be due to the rapidity of growth of the spring plants, which is known to be favourable to the development of virus diseases. The other two facts cannot easily be dealt with in terms of the plant but, as only one season has elapsed, they might be due to some special condition peculiar to particular series of experiments. The three taken in conjunction do, however, suggest the possibility of a seasonal variation of the virus which is the explanation best agreeing with all the data so far accumulated, but this will require several seasons to establish.

SUMMARY.

1. The source and general character of three new virus diseases occurring in *Hyoscyamus* are described under the names of *Hyoscyamus* virus (Hy.) II, III and IV. They have a host range of various solanaceous plants not including any variety of potato.

2. Hy. II and III are non-filterable (through L 3) and are transmitted to and from all their hosts except tomato by the aphid *Myzus persicae*. Hy. III survives for a relatively short period as clarified juice. Hy. II and Hy. III have many characters in common and are probably closely related.

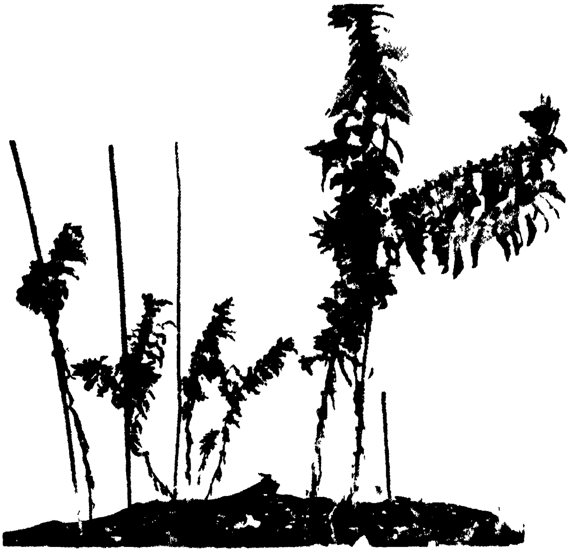

Fig. 1.

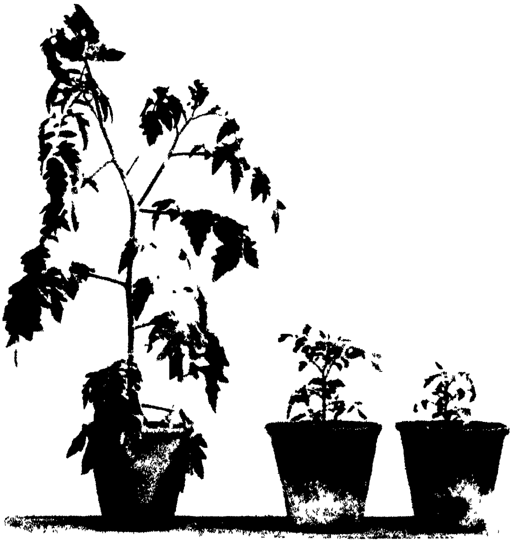

Fig. 2.

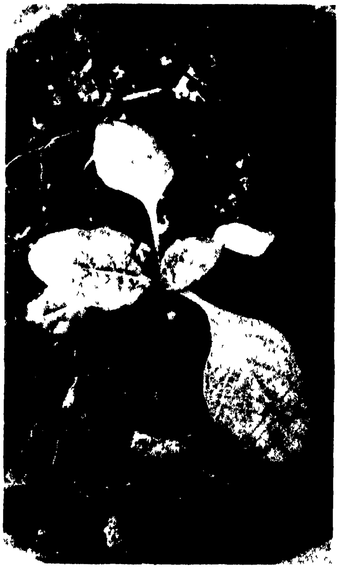

Fig. 3.

HAMILTON.—On Three New Virus Diseases of *Hyoscyamus niger* (pp. 550–567).

Fig. 1.

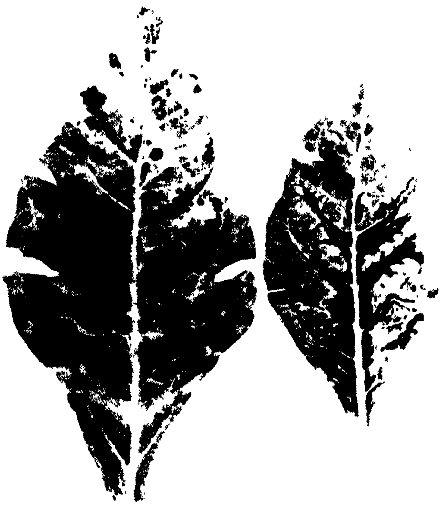

Fig. 2

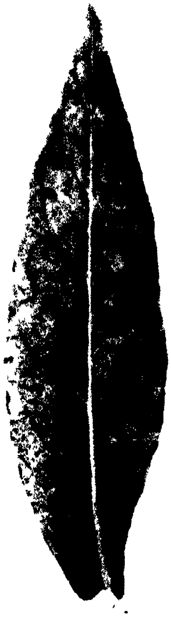

Fig. 3.

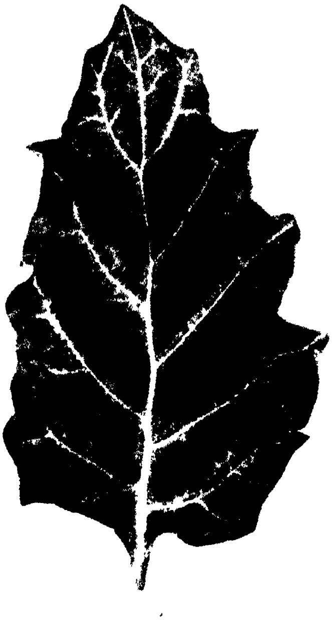

Fig. 4.

HAMILTON.—On Three New Virus Diseases of *Hyoscyamus niger* (pp. 550–567).

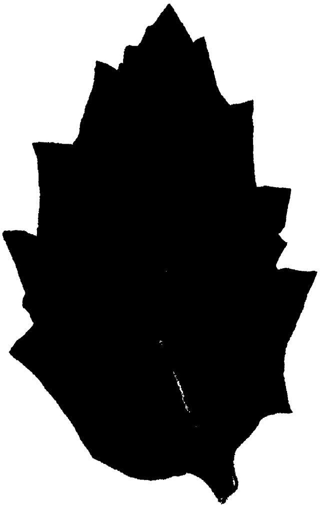

Fig. 1.

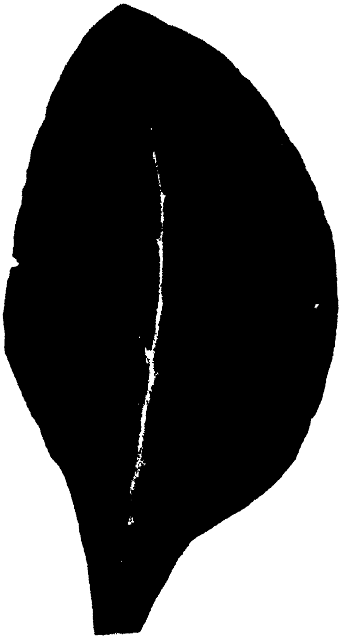

Fig 2.

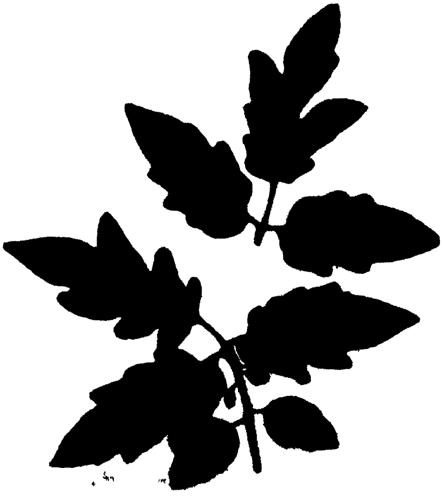

Fig. 3.

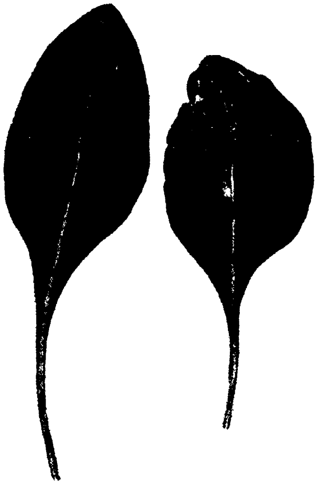

Fig. 4.

HAMILTON.—ON THREE NEW VIRUS DISEASES OF *HYOSCYAMUS NIGER* (pp. 550–567).

3. Hy. IV is a different type of virus, filterable through an L 3 candle and no insect vector has yet been found for it.

4. Problems arising from consideration of the data so far accumulated are submitted and discussed.

This work was carried out under the auspices of the Empire Marketing Board.

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EXPLANATION OF PLATES XXVIII—XXX.

PLATE XXVIII.

Fig. 1. Hy. II in *Hyoscyamus* plants brought in from field as winter buds. Infection was obtained from the two small plants, not from the large one. Height of large plant, about 3 feet.

Fig. 2. Hy. III in tomato, two infected plants and one control of the same age. Height of medium plant, 19 cm.

Fig. 3. Young *Hyoscyamus* plant showing preliminary symptoms ("Clearing of the veins") of Hy. III.

PLATE XXIX.

Fig. 1. Hy. III in *Hyoscyamus* typical late symptoms; compare with similar type of symptom in tobacco.

Fig. 2. Hy. III in tobacco, typical late symptoms. Length of leaf, 15 and 18 cm.

Fig. 3. Hy. II in tobacco, compare with Fig. 4. Length of leaf, 21 cm.

Fig. 4. Hy. II in *Hyoscyamus*; note the puckered bands along the sides of the vein. Length of leaf, 15 cm.

PLATE XXX.

Fig. 1. Hy. IV in *Hyoscyamus*, "pepper and salt" mottle. Length of leaf, 14 cm.

Fig. 2. Hy. IV in tobacco, after passage through tomato. Length of leaf, 23 cm.

Fig. 3. Hy. IV in tomato.

Fig. 4. Hy. IV in *Hyoscyamus*, after passage through tomato. Length of leaves, 8–6½ cm.

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